THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

VOL. CI—NO. 3

Issued August 1, 1932

BALTIMORE, U. S. A.

1932

Entered as second-class matter, August 18, 1914, at the Post Office in Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918.

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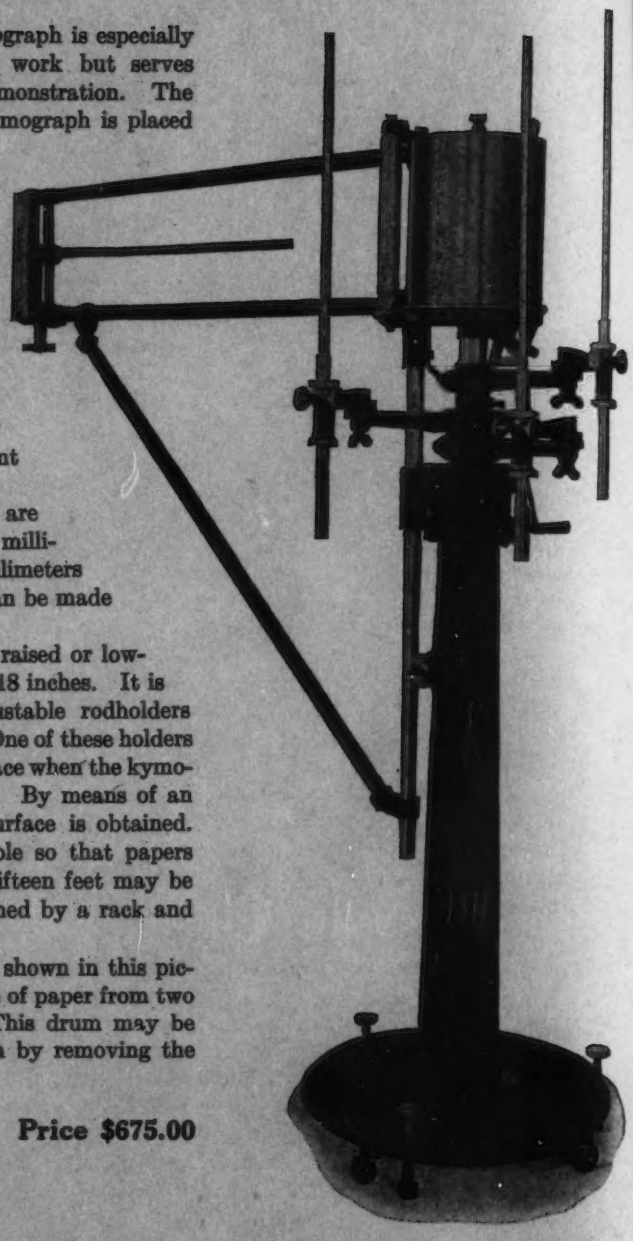

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 101 AUGUST 1, 1932 No. 3

SOME CRITERIA OF ACCURACY FOR THE MEASUREMENT OF THE OSMOTIC PRESSURE OF COLLOIDS IN BIOLOGICAL FLUIDS

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Received for publication May 7, 1932

The study of the osmotic pressure of the colloids of biological fluids has, in recent years, cast much light on the mechanisms which are involved in the maintenance of the water balance of the body. The need for accurate measurements of the osmotic pressure of the various body fluids has been recognized in theory, but in practice the determinations have been carried out by such a variety of methods that it is not surprising to find considerable discrepancies in the pressure values obtained by different investigators from measurements on similar fluids. For example, two investigators (1), (2), employing identical membranes, have published widely different average values for the specific osmotic pressure (pressure per gram per cent protein) of the colloids of human blood plasma. The confusion which has arisen as the result of such discrepancies could be resolved by assuming that the osmotic pressure of colloids of any given biological fluid is a quantity so indefinite, so susceptible to slight variations in the conditions under which the determination is carried out, as to provide practically no information of value for the quantitative study of the processes of osmosis as they occur in the animal body. On the other hand, the experience in this laboratory would seem to indicate that the confusion lies not in the variability of the osmotic pressure itself but in the difficulty of controlling the experimental conditions in such a way that a true osmotic equilibrium may be observed.

It is the purpose of this communication to suggest certain criteria of an osmotic equilibrium and to present details of the methods of procedure which have been devised to meet the demands of these specific standards of accuracy, as they have been developed in the course of investigations to be described in subsequent reports. It is hoped that our experience may encourage others to undertake measurements of the osmotic pressure, for,

when proper precautions are observed, it is not a difficult matter to obtain consistent and apparently entirely reliable results.

Criteria of accuracy in the measurement of the osmotic pressure. The properties of the membrane. 1. The membrane shall be sufficiently permeable, sufficiently thin, and of sufficient area in relation to the volume of the sample to allow an equilibrium to be established as quickly as possible between the diffusible constituents of the inner and outer solutions of the osmometer. When the membrane is too "tight" the temporary pressure due to the osmotic activity of these diffusible constituents may be so high and may persist so long that osmotic equilibrium will never become established; for after 24 hours it is often found that spontaneous changes will have occurred in the colloidal state of the solution. Such changes, once started, will usually continue without interruption and in such a manner that the osmotic pressure will fall continuously to zero.

In the study of biological fluids it becomes necessary to demonstrate that the permeability of the membrane remains essentially unaltered by contact with these fluids. Mucus and fats are found, for example, to decrease the permeability of collodion membranes to such an extent that the time for the attainment of osmotic equilibrium becomes definitely prolonged. If the equilibrium is not attained within 24 hours or less the measurement is apt to be of little value, for the reasons given above.

2. While the membrane must be "rapid" it must nevertheless be strictly impermeable to the colloid under investigation.

a. Membranes, if made from collodion, must be water-clear rather than opaque or even slightly "milky" in appearance, for the latter condition is *prima facie* evidence of irregularities in permeability.

b. The specific permeability of each membrane should be determined and its value should lie within the range known, as the result of preliminary experiments, to be suitable for the particular biological fluid under investigation. The range of specific permeabilities of the membranes used should be reported in connection with all published values for the osmotic pressure of colloids.

c. Ultrafiltrates prepared with such membranes should show no trace of the colloid whose pressure is to be measured.

d. After each determination the outer solution should be examined by applying a sensitive qualitative test for the colloid in question, in order that the possibility of leakage of the solution through the membrane or through the junction of membrane and osmometer may be ruled out. The sensitivity of the test should be determined and the figure representing the greatest dilution of the colloid for which the test is sensitive should be published.

e. All determinations of the osmotic pressure should be discarded in which there is the slightest evidence of the presence of the colloid in the outer solution.

f. No osmometer should be used which does not readily allow the application of such a test to the outer solution.

3. The membranes should be free from gross contamination with bacteria or molds.

a. It is preferable to use a freshly prepared membrane for each determination.

b. When membranes must be preserved before they are to be used it is essential to employ an antiseptic which will not alter the permeability.

c. Antiseptics are apt to cause precipitation or other changes in the colloidal state of the sample. Since this factor is difficult to control, it is advisable to wash the membrane free of all antiseptic material before it is used.

The properties of the colloidal solution. 1. There should be no evidence of changes in the colloidal state of the sample during the determination.

a. There should be no visible evidence of precipitation of the material or of adsorption onto the surface of the membrane.

b. Bacteriological or other tests for invisible changes may be carried out as desired, but it is our experience that, if the determination can be completed within 12 to 24 hours, there is little likelihood of such changes causing any appreciable error. As emphasized above, it is the spontaneous change which may occur in a bacteriologically sterile medium which produces the greatest errors.

c. Spontaneous changes in the colloidal state, often (but not always) accompanied by a visible precipitation of the material, will occur much more quickly at a temperature of 0°C. than at higher temperatures (15°C. to 38°C.). It would therefore seem desirable to carry out the determinations at room temperature, for the spontaneous changes of lower temperatures and the more rapid bacterial changes of higher temperatures are thus avoided.

The properties of the outer solution. 1. Theoretically an ultrafiltrate prepared from the colloidal solution in question would allow the most rapid and most accurate determination of the osmotic pressure. In practice it is found that the time for the attainment of equilibrium is not reduced by the use of ultrafiltrates prepared in the usual manner. Until this question has been further investigated we may assume that in the actual process of filtration, as ordinarily carried out, certain of the relatively non-diffusible constituents are concentrated in the solution and that the filtrate is not a true ultrafiltrate.

For mammalian fluids it has been found that 0.9 per cent NaCl solution is entirely satisfactory, and that with its use the same equilibrium is reached in the same time as with "ultrafiltrate" or Locke solution.

2. The volume of the outer solution should be as small as possible in relation to the volume of the inner solution, in order that the changes in

composition of the latter, resulting from the outward diffusion of certain of its constituents, may be as slight as possible.

Criteria of osmotic equilibrium. 1. In order to be certain that a true osmotic equilibrium has been attained the volume of the solution, as judged by the height of the meniscus in the capillary tube of the osmometer, must be observed to remain absolutely constant, at constant pressure and constant temperature, for a considerable length of time. Depending on the permeability of the membrane and other factors the observation may have to be continued for minutes, hours, days, weeks or even for several months in order to be certain that one has to deal with a true equilibrium. The occurrence of any detectable change of volume during this time should be grounds for discarding the determination. Unfortunately, to insist on this simple but essential criterion of an osmotic equilibrium is to insist that the majority of the figures which have appeared in the literature are unreliable.

a. The temperature of the osmometer should remain constant within a few hundredths of a degree in order that the volume of the contents may remain constant; otherwise with changes in temperature irregular movements of the meniscus will occur which are misleading.

b. Volume changes due to osmosis or ultrafiltration should be reduced to the absolute minimum, for the changes in concentration of the colloid produced at the surface of the membrane by the movement of fluid through the membrane have been found to give rise to changes in the osmotic pressure which may be considerable and which may require many hours for readjustment. For this reason it is desirable that an accurate balance of pressures be maintained by frequent adjustment of the external pressure which is applied to the solution by means of a water manometer.

c. The osmometer tube should have a bore which is sufficiently narrow to favor ease of observation of movements of the meniscus. However, the smaller the bore, the greater is the correction for the capillary rise of fluid in the tube. Consequently the error of the determination of the capillarity should be taken into account in relation to the magnitude of the osmotic pressure to be measured. For the measurement of pressures above 100 mm. water a narrow bore (0.5 mm. or less) is permissible; while for the estimation of lower pressures the tube should be relatively wide (1 to 2 mm.).

d. For the particular osmometer and for the standard membranes used in this laboratory, osmotic equilibrium should be considered as having become established only when the meniscus of the fluid in the osmometer tube has been observed to remain steadily in one position, within a few hundredths of a millimeter for at least 2 hours. At the end of this time it must be demonstrated that a change of 0.5 to 2.0 mm. of water of the pressure applied will, within 5 minutes, cause the meniscus to move for a

measurable distance and at a steady rate, either up or down, according to the direction of pressure change.

e. Determinations of the osmotic pressure should be carried out in duplicate whenever sufficient material is available, and the values obtained in the duplicate determinations should not differ by more than the greatest error of determining the correction for capillarity. This error should rarely exceed 10 per cent of the total capillary correction.

DETAILS OF THE METHOD USED IN THIS LABORATORY. *The osmometer.* The micro-osmometer of Krogh (3), (4) possesses the unique advantage of requiring relatively small amounts of biological fluids for the accurate determination of the osmotic pressure of colloids. A sample of from 0.2 cc. to 0.7 cc. is sufficient, and the capacity of the osmometer may be adjusted in each case to the volume of the sample available, within the limits just given. The osmometer possesses the theoretical advantage of allowing the outer solution, the volume of which is not greater than 0.1 cc., to come into equilibrium, so far as diffusible substances are concerned, with the sample, a result which may be accomplished with minimal changes in the composition of the latter. Furthermore, by this method an osmotic equilibrium is actually attained. The so-called dynamic methods, so much in use at the present time, will, unless they are employed under almost ideal conditions, often lead to gross errors. Finally, with the Krogh osmometer it is possible to test the outer solution for the presence or absence of proteins as a check on the strict semi-permeability of the membranes with respect to the substance whose osmotic pressure is being measured.

The one disadvantage of the method, as originally described, has been the difficulty of preparing suitable membranes. This difficulty has been largely overcome in this laboratory during the course of experiments in which it was desired to measure the osmotic pressure of blood serum and lymph of dogs.

Preparation of membranes. The collodion solution is prepared by dissolving 8 grams of dry "Parlodion" shreds for each 100 cc. of solution in equal volumes of absolute ethyl alcohol and anhydrous ether. As it requires several days for the collodion to become completely dissolved it is advisable to prepare a stock solution of 500 cc. or more. The solution should be prepared and preserved in a glass stoppered vessel.

In the original method the sacs are formed on glass capillary tubes of approximately 4 mm. outside diameter, and having rounded tips. These tubes were filled with mercury to facilitate removal of the membranes. I have considered it desirable to omit the use of mercury, due to the fact that droplets of this material almost invariably become enclosed in the collodion at the tip of the sac. Oxidation of this mercury in the presence of ether leads to the formation of a reagent which may precipitate serum

proteins. With a stopcock sealed to the upper end of the glass tube, air may be trapped in the bore in such a way that, on dipping the tip of the tube into collodion two or three times, with intervals for drying of 15 minutes or longer, a hard cap may be formed which will prevent the flow of collodion into the bore of the capillary during the formation of the membrane.

Whenever it is desired to make up a batch of membranes, a 50 cc. glass-stoppered cylinder is filled to the mark with fresh collodion from the stock solution. One of the glass capillary tubes is dipped into the solution in the cylinder to a depth of about 8 cm. It is then raised until only the tip remains in contact with the surface of the solution, and the excess collodion is allowed to drain for exactly 90 seconds. The atmosphere of alcohol and ether vapors present in the upper part of the cylinder will prevent drying, thus facilitating a free and uniform drainage. The tube is then removed and allowed to dry in a vertical position, at room temperature, for 60 seconds. It is then dipped a second time and allowed to drain for 120 seconds, whereupon it is immediately placed, still in the vertical position, in a drying chamber through which air is constantly flowing. Such a chamber may be made from a 5 pound ether can by cutting in the top a number of holes of a diameter slightly greater than that of the glass tubes. The air is led in through the central spout of the can, and is conducted to the bottom through a glass tube, whence it escapes to diffuse upward, past the membranes, and out through the holes. The air coming from the pressure supply is passed through concentrated sulphuric acid to remove water vapor, through soda-lime to remove acid spray, through a filter of cotton gauze to remove lime dust, and finally through ethylene glycol. The trace of glycol present in the air keeps the membranes pliable enough so that they can be removed from the glass tubes after all the alcohol and ether has been allowed to evaporate. For complete drying approximately 24 hours are required.

Some care is required in the removal of the dry sacs from the glass tubes. The sac is first cut through at a distance of approximately 4 cm. from the tip. The stopcock is opened to allow the membrane to fill with air while it is being removed. The tip is then loosened by applying a heavy, twisting pressure with the thumb and forefinger. The membrane should then be slowly pushed off, pressure being applied just back of the tip. In order to prevent the membranes from becoming soiled or greasy it is a good idea to cover the fingers with several layers of gauze. Should it prove to be impossible to remove the sac at the first attempt it will be necessary to replace it in the drying chamber for several hours in order that it may become further softened by the deposition of glycol. The sacs so prepared are hard, dry and transparent, presenting none of the milky appearance of the "wet" membranes prepared in the ordinary way. The least trace

of water vapor in the air will cause the "milky" precipitation of collodion as it dries, with the consequence that marked irregularities in permeability will occur.

The membranes are now placed in a tightly stoppered jar containing a solution prepared by adding 5 cc. of water to 95 cc. of absolute ethyl alcohol. It has been found by trial that this concentration of alcohol will, in 24 hours or less, cause the dry membranes to swell to the proper degree of permeability. The discovery of this general method of treating dry membranes to render them permeable is attributed to Brown (5).

After being allowed to swell in alcohol the membranes are rinsed many times, inside and out, in distilled water, and preserved in 0.9 per cent NaCl solution saturated with chloroform. It has been found that the acriflavine used by Krogh as an antiseptic is adsorbed onto the membranes, rendering them brittle and increasing their permeability in an irregular manner. The chloroform does not have this effect, but it possesses the disadvantage that it will precipitate protein from the serum. Consequently it must be removed by thorough rinsing in fresh saline before the membrane is to be used.

Standardization of membranes. The permeability of the membranes used has been repeatedly tested by the determination of the rate of filtration of water. This method of standardization has proved helpful in the hands of Zsigmondy (6), Krogh (4), and others. However, as these authors have not included in their calculation of the filtration rate the factor of the thickness of the membrane, their "filtration numbers" are of little value to anyone else. In order that the membranes used by different investigators may be compared it would be desirable to adopt some definite standards for the measurement of permeability. In the absence of such an accepted standard I have chosen to define as a measure of the "specific permeability" of a membrane, the number representing the $cm.^3$ of water filtered per second through 1 $cm.^2$ of membrane of 1 cm. thickness, by a hydrostatic pressure difference of 1 atmosphere (1 atm. = 760 mm. Hg) at a temperature of 20°C.

The filtration rate for water has been determined usually at average pressures of from 200 to 300 mm. of mercury. The sac is connected to a 1 cc. pipette, calibrated in hundredths of a cubic centimeter which replaces the osmometer tube of the usual set-up. Pressure is applied by air transmission from a mercury manometer. The time required for the filtration of a definite volume of water is determined, as is the mean pressure during this time. In calculating the area of the membrane the tip, which is relatively dense and thick, is neglected. For the measurement of the average thickness a micrometer gauge is employed which reads to 0.01 mm., and the membrane is measured while wet. The tip of the membrane is cut off and it is slit lengthwise. The thickness of the membrane is

measured at each end and in the middle and a figure is chosen which seems to express as nearly as possible the mean thickness. As this measurement involves the destruction of the membrane it must of course be carried out either after the membrane has been used for a determination of osmotic pressure, or on a sample membrane which is sacrificed for the purpose of obtaining an idea as to the probable permeability of the other membranes of the same series. It very rarely happens that significant variations will occur in any given group of membranes, prepared at the same time and with due regard for uniformity of treatment. Where v is the volume in cubic centimeters filtered in t seconds at a mean pressure of p atmospheres through a membrane whose area is a square centimeters and whose thickness is h centimeters, the specific permeability is $P = \frac{vh}{atp}$. Membranes prepared as directed should possess permeability numbers lying in the range of 20×10^{-8} to 40×10^{-8}. However, accurate and sufficiently rapid determinations can be carried out on serum or lymph with membranes whose permeabilities vary between 10×10^{-8} and 50×10^{-8}. The membranes should not be thicker than 0.04 to 0.08 mm.

Should it be found by trial that membranes made by this method are either too permeable or not permeable enough it is only necessary to vary slightly the concentration of alcohol used for swelling them. The permeability will be increased by a higher concentration and, conversely, will be decreased by a lower concentration of alcohol.

It has been found that membranes softened in a solution consisting of 98 volumes of alcohol and 2 volumes of water are distinctly permeable to the serum proteins. With one such membrane, whose specific permeability number was 340×10^{-8}, the pressure continued to fall for 20 hours, without a true equilibrium becoming established. On the other hand, membranes softened in a solution consisting of 90 volumes of alcohol and 10 volumes of water are so tight that it may require from 12 hours to several days for the attainment of a true equilibrium. The permeability numbers of these membranes were not determined. When the equilibrium pressure could be measured with these membranes the value was found to be the same as was obtained with the more permeable membranes recommended above. The use of such tight membranes is to be avoided, for the pressure falls so slowly toward the equilibrium that one is very apt to be misled and to record pressure values which are too high. Even with scrupulous care, it may not be possible to obtain the correct equilibrium, for in the time necessary to attain this state the proteins of the serum will frequently undergo spontaneous changes which will cause a continuous falling of the pressure below the original equilibrium value. In fact the pressure may never reach a steady state, but often falls gradually to zero in the course of 4 or 5 days.

The difficulties inherent in the use of tight membranes will obviously appear whenever a membrane, originally of the correct permeability, becomes "plugged" by the adsorption of fat, mucus, or other material contained in certain types of biological fluids. For example, samples of bile which contain considerable quantities of mucus may exhibit pressures up to 1 atmosphere, which will fall off very slowly during the course of 4 or 5 days. Putrefaction or precipitation of the material may occur before the pressure has fallen lower than 0.5 atmosphere. The membranes used may originally be readily permeable to bile salts and even to serum proteins, but after contact with the mucus of bile the specific permeability may be reduced to such an extent that it becomes impossible to determine whether or not bile exhibits a true osmotic pressure of colloids. When the bile is aspirated from the gall bladder of the living animal, however, it often contains relatively little mucus, and under these conditions the osmometer pressure rapidly falls, within 24 hours, to atmospheric, and the specific permeability of the membrane shows relatively little change. The relatively high content of fat in the intestinal lymph of animals fed on cream may decrease the permeability of the membrane in a similar manner so that the time necessary for the attainment of osmotic equilibrium will be considerably prolonged, as compared with the usual time required for serum or for lymph of low fat content.

The time necessary for the attainment of osmotic equilibrium may also be unduly prolonged, with the usual possibilities of error in the measurements, when the membranes employed are too thick. A membrane which possesses the correct specific permeability may nevertheless be impractical if its thickness so retards diffusion between the inner and outer solutions of the osmometer that an equilibrium cannot become established before bacterial or other changes in the proteins have altered the colloidal state of the material.

The determination of the osmotic pressure. The details of the construction and the general method of use of the osmometer will not be described as they have been adequately treated in the article by Krogh and Nakazawa (2). Certain suggestions, coming out of an actual experience with the method may, however, be in order.

A Dewar flask (pint size "thermos" bottle filter) may be used in place of the more expensive and less practical thermostat bath. This flask is filled with water at room temperature. Two osmometers, supported by a suitable metal bracket, may be placed in each flask.

The bore of the osmometer tube may be reduced to less than 1.0 mm., the size recommended by Krogh. This provides for more rapid changes in the level of the meniscus.

Although a cathetometer or other form of telescope provided with a cross-hair is desirable for the observation of the movements of the meniscus,

it becomes necessary to provide one such instrument for each osmometer in use at any one time. The expense of such a series of telescopes may be avoided if a cross-hair is supported directly against the osmometer tube. This may be done by cementing a hair across a metal clip, such as is provided with the glass tuberculin syringes in common use. This clip is provided with a spring which will support it against the osmometer tube. The clip is moved up or down as the level of the meniscus changes. By observation with a pocket lens the direction of movement of the meniscus with respect to the cross-hair may be detected.

The pressure of a water manometer is required, in addition to that of the column of fluid in the osmometer, to balance the osmotic pressure. I have found it desirable to leave the connection to the manometer open from the very beginning of the determination. The pressure is adjusted from time to time in such a way as to prevent any appreciable volume change of the fluid in the osmometer. This precaution is essential, for it has been found that if too great a pressure is applied there will occur, as a result of the ultrafiltration, an increase of the concentration of protein at the surface of the membrane sufficient to raise the osmotic pressure considerably above its equilibrium value. Conversely, if the applied pressure is too low the concentration at the surface will be lowered and too low an osmotic pressure will be registered. In either case it may require from 2 to 12 hours for a complete restoration of the equilibrium conditions. It should be noted, however, that even when the volume of the solution is kept constant the initial pressure necessary to balance the system will be considerably higher than that required at equilibrium. This temporary high pressure is undoubtedly due to the presence in serum of slowly diffusing substances which exert an "osmosis" pressure until they have diffused into the outer solution.

The true osmotic equilibrium should be established within 1 to 6 hours. In some instances the equilibrium pressure is found to remain constant to within 0.5 mm. of water for 4 to 5 days. In most cases the pressure will fall off very gradually, from 5 to 15 millimeters a day. This fall is presumably due to spontaneous changes in the proteins, for the same phenomenon can occur in the absence of bacterial contamination or passage of protein through the membrane. Occasionally a distinct precipitation of protein will appear as the result of bacterial action but this seldom occurs in less than 48 hours. Equilibrium is not considered as having become established until the balancing pressures check within 1 or 2 mm. of water for at least 2 hours. When determinations are carried out in duplicate a difference of more than 5 mm. in the two determinations is exceptional.

After the determination has beem completed the outer solution should be tested for proteins. The nitric acid ring-test will readily detect the

presence of serum protein at a dilution of 1:40,000. The result of this test should be negative if the determination is to be considered reliable.

In order to determine the correction for the capillarity of the osmometer tube it is necessary to take the mean of the heights attained by the serum in falling and in rising toward the equilibrium level. This is necessary because, in the case of a capillary of narrow bore, the serum may appear to have come to rest when actually it is still 5 to 10 millimeters from the point of equilibrium.

TABLE 1

SAMPLE	TOTAL PROTEIN	OSMOTIC PRESSURE OF COLLOIDS	MEAN SPECIFIC OSMOTIC PRESSURE	SPECIFIC PERMEABILITY OF MEMBRANES USED	PERIOD OF OBSERVED EQUILIBRIUM. TIME FROM START
	per cent	*mm.* H_2O	*mm.* H_2O	*cm.*^{3} *per sec. per atm.* $\times 10^{-6}$	*hours*
Dog serum	4.65	182	40	22	2 to 4
		187		22	2 to 5
Dog serum	5.75	252	44	26	3 to 8
		250		32	4 to 6
Dog serum	6.41	258	40	51	3 to 25
		256		31	2 to 4
Dog serum	6.68	276	41	36	6 to 120
Dog serum	6.79	275	40	37	3 to 20
		271		41	2 to 20
Dog serum	6.86	325	48	22	2 to 5
		330		18	3 to 7
Dog serum	7.12	329	46	21	2 to 4
		330		32	2 to 5
Human serum	7.50	370	49	35	5 to 7
		366		37	3 to 10

Table 1 presents typical examples of the results obtained by the use of the Krogh osmometer. By reference to the last column of the table it will be noted that most of the experiments were terminated at the end of 4 to 8 hours. However, in several instances the osmotic equilibrium was observed to remain absolutely constant for a much longer time. It is believed that the data will justify the choice of a two-hour period of observation of a constant pressure as a sufficiently rigorous test of the attainment of the true osmotic equilibrium.

For further examples of results obtained by the method the reader is referred to the next article in this journal (pp. 421 to 433).

SUMMARY

1. The occurrence of considerable discrepancies in published values for the osmotic pressure of colloids of similar biological fluids indicates the necessity for the adoption of standard criteria of accuracy for such measurements.

2. Definite criteria are suggested.

3. Details of the procedures which have been evolved to meet the demands of these criteria are presented.

a. A method for preparing collodion sacs of the proper degree of permeability for the determination of the colloid osmotic pressure of serum or lymph is described.

b. A standard designation for the "specific permeability" of membranes is proposed and the method of standardization described.

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THE CONCENTRATION AND OSMOTIC PRESSURE OF THE PROTEINS IN BLOOD SERUM AND IN LYMPH FROM THE LACTEALS OF DOGS

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Received for publication May 7, 1932

The lymph obtained from the thoracic duct of a resting animal is presumably derived, for the most part, from the liver and intestines (1). In order to exclude the lymph from the liver and thereby to obtain from the thoracic duct a so-called "intestinal" lymph, various workers have employed operative procedures such as ligation of the lymphatics of the liver (1) or removal of this organ (2). Such procedures are difficult to carry out and may not be entirely reliable, for they necessarily involve manipulation of the splanchnic organs to an extent which may well result in the production of marked changes in the composition of the lymph from the intestine. It has been found in this laboratory that a true intestinal lymph can be obtained by direct aspiration from the lacteals of the mesentery, a procedure which was developed in the course of an investigation undertaken primarily for the purpose of determining the relation of the absorbing force of the intestine to the osmotic pressure of the colloids of the intestinal lymph (3). During this investigation the concentration and osmotic pressure of the proteins of this lymph, as well as of blood serum from the same animals, were determined, and the mesenteric venous pressure was measured in the majority of animals. Analysis of these data elicits information that is obviously more pertinent to the question of the normal mechanism of lymph formation in the intestine than is provided by studies carried out on lymph from the thoracic duct. The results of this study of the intestinal lymph indicate that the protein content of the lymph is determined within fairly definite limits by the protein content of the serum; that the osmotic pressure for one per cent of protein of the lymph is of the same magnitude as that of serum; and that the mesenteric venous pressure is sufficiently high, in relation to the calculated "effective" osmotic pressure of the blood, to account for the continuous formation of intestinal lymph by a process of rapid filtration of protein-containing fluid through the capillaries.

METHODS. *Collection of lymph from the lacteals.* Although it is possible to collect lymph from a fasting animal, a good deal of massage of the

gut is necessary in order to obtain a large enough sample. It is probable that the lymph obtained in this way will differ in composition from the normal intestinal lymph, for as the result of handling the gut, vasomotor changes as well as changes in the permeability of the capillaries may be presumed to take place. When the animal is in the digestive state, however, the lacteals of the intestinal wall and of the mesentery are so well filled that it is possible to obtain the desired amount of lymph with a minimum of manipulation of the gut. Consequently in each instance the animal has been fed a large meal of meat, bread and skimmed milk, in order to insure an adequate filling of the lacteals with the digestive lymph. Enough food was given in the evening so that an excess remained in the cage the following morning.

The dogs were anesthetized by the intravenous injection of 0.3 gram of sodium barbital per kilo of body weight. When the abdominal cavity had been opened the lymphatics of the mesentery were usually found to be well filled with a milky lymph.

The lymph was collected by introducing a 24 gauge hypodermic needle into the lymphatic vessels of the mesentery of the jejunum. The lymph was aspirated by gentle suction into a test tube, on the walls of which a thin layer of sodium oxalate had been precipitated by evaporation. Suction was provided by appropriate connections to a filter pump and to a pressure regulator which provided a constant negative pressure of approximately 7 cm. of water. The needle was inserted in turn into several of the larger lymphatic trunks. By rolling the gut lightly between the fingers it was usually possible to obtain 0.1 cc. or more at each puncture. No attempt was made to exhaust the available supply. When the lymph ceased to flow freely the needle was withdrawn and inserted into another lymphatic trunk. Proceeding in this manner it was relatively easy to collect from 0.5 cc. to 1.5 cc. of lymph from the upper 30 to 40 cm. of jejunum. From 0.3 to 0.5 cc. of the sample was sufficient for an osmotic pressure determination. The remainder was used at once for protein analysis. After the osmotic pressure had been determined the lymph was removed from the osmometer sac and used for the remaining chemical analyses.

In all cases the lymph has been milky-white in color and therefore apparently free from contamination with blood.

Measurement of the mesenteric venous pressure. In most instances an interval of from 1 to 3 hours elapsed between the collection of lymph and the measurement of the venous pressure. This interval of time was required for the determination of the absorbing force of the gut, a procedure which required that a loop of jejunum, in the region from which lymph had been collected, be isolated according to the method which has been described elsewhere (3). For the measurement of the venous pressure a

hypodermic needle connected to a manometer containing physiological salt solution was inserted into a side branch of a mesenteric vein of the isolated loop. The needle was directed centrally so as to give readings which represent as nearly as possible the lateral pressure in the nearest connecting vein. The measurement was repeated several times. In a few instances the pressure was also measured before and again immediately after the collection of lymph. The values so obtained were approximately the same as those found after the absorption period.

For the measurement of the venous pressure the same level of reference was chosen as was adopted for the determination of the absorbing force, viz., the plane of the horizontal diameter of the lumen of the isolated loop.

Collection of blood samples. Blood for the determination of the concentration and osmotic pressure of proteins was withdrawn from the heart immediately after the measurement of the venous pressure. The blood was allowed to clot, centrifuged at high speed for 10 minutes, and the serum removed at once.

Determination of the total protein and albumin and globulin fractions of serum and lymph. The protein concentrations of the various fractions were calculated from direct nitrogen determinations by the micro-Kjeldahl aeration method of Folin and Farmer (4). For the titrations, freshly prepared and frequently standardized solutions of 0.01 N NaOH and HCl were used. Blank determinations were carried out on the reagents and the correction found to be negligible. The accuracy of the method was checked by the analysis of various dilutions of a standard urea solution. The greatest error of the method as used appears to be of the order of 0.1 gram of protein per 100 cc. For the total nitrogen determinations 0.1 cc. of serum or lymph was used, for the accurate measurement of which special 0.1 cc. pipettes were employed. Duplicate analyses were carried out in almost every instance.

For the separation of the globulin fraction the method of Howe (5) was employed, using 19 volumes of 22.5 per cent solution of Na_2SO_4 to 1 volume of the sample. The mixture, so prepared, was allowed to stand in the incubator at 38°C for 12 hours in order to insure the complete flocculation of the globulins. The presence of fat in the lymph seemed to favor rather than hinder the separation. In almost every instance a water-clear filtrate was obtained for the analysis of the albumin nitrogen. The globulin was calculated by difference.

The determinations of non-protein nitrogen were carried out on tungstic acid filtrates of serum and lymph, prepared according to the standard method of Folin. Such determinations were not carried out in the seven earlier experiments. The figures for these experiments have therefore been arbitrarily corrected by subtracting from the values for total nitrogen and for albumin nitrogen of lymph and of serum respectively corrections

which are the approximate averages of values for non-protein nitrogen found in the seven later experiments. These corrections are, for lymph 0.3 per cent and for serum 0.22 per cent, calculated as protein. Such a correction of the figures for the protein of serum is completely justified by the fact that the non-protein nitrogen content of serum is a relatively invariable quantity. Thus the seven determinations which were carried out gave values of 0.26, 0.22, 0.22, 0.22 0.21, 0.22, and 0.23 per cent, as protein. In the case of lymph the values found in seven determinations were 0.15, 0.25, 0.31, 0.28, 0.51, 0.42 and 0.35, with an average of 0.324 per cent, calculated as protein. The variation is greater than was the case for serum. Nevertheless, the error which is introduced by accepting an average value of 0.3 per cent for the non-protein nitrogen of lymph, calculated as protein, is not sufficiently great to invalidate the conclusions which have been drawn from the figures as given.

The determination of the osmotic pressure of colloids of serum and lymph. Details of the method are described elsewhere in THIS JOURNAL (6).

In no experiment was enough lymph collected to allow the determinations to be carried out in duplicate. In all cases a cathetomoter was used for observation of the movements of the meniscus in the osmometer tube. By the aid of this instrument movements of less than 0.01 mm. can readily be detected. Only when the meniscus had remained absolutely steady for 2 hours or longer was it considered that a true osmotic equilibrium had been attained. In several instances the equilibrium pressure was found to remain unchanged for as long as 48 hours. During this time a change of from 0.5 mm. to 2.0 mm. of the pressure applied by means of the water manometer was sufficient to cause, within 5 minutes or less, a definite movement of the meniscus. In most instances it was possible to complete the determinations within 5 to 10 hours.

In order to determine whether the sodium oxalate used to prevent clotting of the lymph would affect the osmotic pressure of colloids, a control experiment was carried out. A pint of cream was administered to a dog by stomach tube in the evening. Next morning the animal was anesthetized with barbital, the thoracic duct cannulated and 20 cc. of lymph collected. The lymph was defibrinated by shaking with glass beads. The sample was then divided into three portions. One portion was completely saturated with sodium oxalate. The second portion was placed in one of the tubes used for collection of lymph from the lacteals, on the walls of which a few milligrams of oxalate had been deposited by evaporation from a saturated solution. The third portion remained untreated. The osmotic pressure of the colloids of each sample was then determined. The values found were successively 171 mm., 185 mm. and 181 mm. It appears that complete saturation with sodium oxalate reduced the osmotic pressure approximately 6 per cent, whereas the amount of oxalate ordinarily

added in the collection of lymph caused no significant change in the osmotic pressure of colloids.

For the determination of the osmotic pressure of serum a single osmometer was set up in each of the first 6 experiments, but in the last 8 experiments duplicate determinations were carried out. The greatest variation between the pairs of values so found was 15 mm. of water: it is unusual to

TABLE 1

The protein content and osmotic pressure of colloids of blood serum and of lymph from the lacteals

Data arranged by experiments in ascending order of the total protein content of the sera. Figures marked with an asterisk have been arbitrarily corrected for non-protein nitrogen, as explained in the section on methods.

NUMBER OF EXPERIMENT	ALBUMIN		GLOBULIN		ALBUMIN / GLOBULIN		TOTAL PROTEIN		PROTEIN RATIO LYMPH / SERUM	SPECIFIC OSMOTIC PRESSURE		TOTAL OSMOTIC PRESSURE	
	Serum	Lymph	Serum	Lymph	Serum	Lymph	Serum	Lymph		Serum	Lymph	Serum	Lymph
	per cent	*per cent*	*per cent*	*per cent*	*ratio*	*ratio*	*per cent*	*per cent*	*ratio*	*mm.* H_2O	*mm.* H_2O	*mm.* H_2O	*mm.* H_2O
12	2.69	1.19	1.96	0.98	1.37	1.22	4.65	2.17	0.47	39.8	32.2	185	70
13	2.50	0.83	2.50	0.84	1.00	0.99	5.00	1.67	0.33	41.0	34.1	205	57
14	2.53	1.09	2.61	0.97	0.97	1.12	5.14	2.06	0.40	37.0	29.1	190	60
3	*3.03		2.29		1.32		*5.32	*2.75	0.52	45.7		243	[110]
7	*3.57	*1.97	2.18	1.07	1.64	1.84	*5.75	*3.04	0.53	43.6	45.3	251	152
4	*3.08	*1.68	3.13	1.10	0.98	1.53	*6.21	*2.78	0.45				
8	2.77	2.14	3.64	1.85	0.76	1.16	6.41	3.99	0.62	40.1	39.6	257	158
5	*3.07	*1.56	3.61	1.64	0.85	0.95	*6.68	*3.20	0.48	41.3	40.0	276	128
2	*2.98		3.80		0.78		*6.78			42.6		289	142
9	3.03		3.76		0.81		6.79	3.93	0.58	40.2	40.1	273	170
10	4.02	2.15	2.84	1.07	1.41	2.01	6.86	3.22	0.47	47.8	43.2	328	139
11	4.39	2.85	2.73	1.70	1.61	1.67	7.12	4.55	0.64	46.3	47.1	330	214
6		*1.82		1.52		1.20		*3.34			37.4		125
1												237	89
Average....	3.18	1.72	2.80	1.25	1.17	1.39	5.98	2.97	0.499	42.3	40.8	256	125

observe so great a discrepancy. The least variation was 1 mm. and the average was 4.5 mm.

The collodion sacs used for the determinations of the osmotic pressure of serum and of lymph in the last 10 experiments were standardized according to their rates of water filtration. The specific permeabilities (6) so determined varied from 18×10^{-8} to 51×10^{-8} cm.^{2} per second per atmosphere.

PRESENTATION AND DISCUSSION OF DATA. *Albumin and globulin fractions.* From an examination of the data, which are presented in table 1,

it appears that the albumin content of the lymph is lower than that of the serum in each case. The same relationship holds for the glubolin fractions. The albumin:globulin ratio on the other hand is higher for lymph than for serum, in most instances. The finding of relatively more albumin than globulin in the lymph is in accord with the observation of Munk and Rosenstein (7) who found albumin:globulin ratios of 4.0 and 2.5 in two samples of lymph from a fistula in a patient. Morawitz (8) who, so far as I am aware, is the only investigator to have previously determined the albumin and globulin fractions of serum and lymph in the same animal, found from the analysis of two samples of lymph from the thoracic duct of dogs ratios which were greater than unity and somewhat higher, in both cases, than the corresponding ratios for serum. The ratios for lymph were 1.56 and 1.58 and those for serum were 1.25 and 1.44. In general the available data support the generally accepted idea (8), (9), (10) that serum albumin, having a lower molecular weight than serum globulin, can pass more readily through the capillary wall.

Ratios of protein of lymph to protein of serum. Although the total protein content of the lymph is lower, in each case, than that of the serum, it appears that with increasing protein concentration of the serum there is an increase in the corresponding lymph protein content. Corresponding to the lowest value for the protein concentration of lymph, 1.67 per cent, we find a serum protein of 5.0 per cent; and for the case of the highest value of lymph protein content, 4.55 per cent, the protein of the serum is present at a concentration of 7.12 per cent. This relationship is brought out more clearly in the column showing the ratio of protein of lymph to protein of serum. The lowest value of the ratio is 0.33, the highest is 0.64, the mean is 0.50 and the average deviation from the mean is ± 0.072. Exactly similar ratios appear, by calculation, from the observations of Loewen, Field and Drinker (10) on serum and on the lymph from the thoracic duct of a series of dogs. From the data on eight animals in which there was encountered a range of protein concentrations for blood of 4.19 per cent to 8.84 per cent, and of lymph from 2.12 per cent to 4.93 per cent, calculation gives the following ratios: 0.55, 0.56, 0.51, 0.39, 0.44, 0.64, 0.45 and 0.30, of which the lowest value is 0.30, the highest is 0.64, the mean is 0.48 and the average deviation from the mean is ± 0.085. In addition, ratios ranging from 0.36 to 0.76, with an approximate average of 0.55 may be found by calculation from the data for normal animals of Meyer-Bisch and Günther (11) who published a large number of figures on the protein content of serum and of lymph from the thoracic duct of dogs. Arnold and Mendel (12) obtained ratios of 0.63 and 0.65 for thoracic duct lymph of two normal anesthetized dogs.

The close correspondence of the ratios of protein of lymph to protein of serum in these series of observations suggests that for a given level of

the serum protein concentration the protein content of the lymph may vary only within certain fairly definite limits, the lower and upper levels of which are, for the normal animal, probably indicated fairly accurately by the lymph:serum ratios of 0.30 and 0.70 respectively. As a corollary to this conclusion it follows that a greater variation in the concentration of the lymph can occur in an animal having a high serum protein content than in one having a low concentration of serum protein. For example, if a dog has a serum protein of 4 per cent the lymph protein is limited to the range between 1.2 per cent and 2.8 per cent, a total variation of 1.6 per cent. An animal with a serum protein of 9 per cent, on the other hand, could presumably form a lymph containing anywhere from 2.7 per cent to 6.3 per cent, a total variation of 3.6 per cent. It may therefore be assumed that the variability of the protein content of the lymph is exactly proportional to the serum protein concentration, the variation being 0.4 per cent of protein for each 1 per cent of serum protein.

Specific osmotic pressure. The figures in table 1 which show the osmotic pressure of colloids for one per cent protein are calculated by dividing the values for the total osmotic pressure of the serum and lymph respectively by the corresponding protein contents. These values of the "specific osmotic pressure" of protein vary from 32 mm. to 48 mm. of water. Contrary to the recent findings of Loewen, Field and Drinker (10) the figures for lymph are, with two exceptions, *lower* than the corresponding figures for serum. In general the difference between the figures for lymph and those for serum may be explained by the fact that the total protein concentration of the lymph is always lower than that of the serum. As pointed out by Krogh (9) the osmotic pressure per one per cent protein falls as the concentration of protein decreases. This author presents in graphic form the relation between these two factors as found by several investigators for human blood sera, the protein concentrations of which were varied by ultrafiltration on the one hand and by dilution with Ringer solution on the other hand. Points plotted onto this graph from my data for lymph and for serum fall within the expected area. Five of the seven values given by Loewen for dog serum also lie within the range of figures determined by these curves, but the figures presented by this author for cervical and thoracic duct lymph cannot be reconciled either with his own figures for dog serum or with my figures for lymph, or with any reliable values for human serum which have appeared elsewhere in the literature. His values for lymph range from 55 mm to 103 mm with an average of approximately 70 mm. of water per one per cent protein, as contrasted with values of 44 mm. to 59 mm. for serum. Furthermore the values obtained by this author for the specific osmotic pressure of lymph vary *inversely* as the protein content of the lymph; i.e., the higher the protein content of the lymph the lower is the osmotic pressure per one per cent of protein. Inasmuch

as the figures given by these authors represent the only data previously published on the colloid osmotic pressure of lymph it will be necessary to discuss their findings in detail in order to discover, if possible, the reasons for the marked discrepancy between their values and the values found in the present study for lymph from the lacteals.

Such an anomalous relationship between concentration and specific osmotic pressure of colloids, as found by these investigators for lymph from the thoracic duct and cervical regions, has been encountered previously, so far as I am aware, only in the case of albumin of the urine of patients suffering from nephritis or cardiac insufficiency (13). Pressures as high as 280 mm. of water for one per cent protein are reported from studies on the urine of such patients. It is assumed that the capillaries of the glomeruli exhibit a selective permeability to the proteins of the serum, allowing protein of small molecular size (and correspondingly high osmotic pressure per gram) to pass through, but retaining the larger molecules, especially the globulins, in the blood. That this assumption is probably correct is indicated by the fact that the serum of such patients, which according to this theory should retain only the larger protein molecules, shows a relatively low specific osmotic pressure as compared to the serum of normal individuals. Loewen assumes a similar selective permeability of the capillary walls to explain the high specific osmotic pressures of lymph samples from the thoracic duct and cervical trunks. As serum albumin consists mainly of smaller and osmotically more active molecules than serum globulin this author suggests that the high osmotic pressure of the lymph must be due to the presence in this fluid of relatively much more albumin than globulin. Unfortunately he does not present chemical analyses to support this assumption. From the data of table 1 it is clear that the albumin: globulin ratio of lacteal lymph is somewhat higher in most instances than the corresponding ratio for serum. However, it is very doubtful that the albumin-globulin ratio of thoracic duct or cervical lymph is ever high enough to account for the high specific osmotic pressures which have been reported. It is probable that all lymph contains a considerable amount of globulin. It is also probably that only a small fraction of the albumin of the serum is capable of exerting as high a specific pressure as has been observed for the case of albuminous urine, for it has been found that edema fluid, which often contains relatively high proportions of albumin, exhibits a specific osmotic pressure which is not higher than that of the blood plasma (13).

The only explanation which can be offered at the present time for the discrepancy between the previously reported figures and those submitted here is that the former do not represent true equilibrium values. Although the authors refer to the difficulties of the method, especially the preparation of suitable membranes, they do not publish the criteria which enabled

them to decide when an osmotic equilibrium had become established. According to the criteria we have laid down in a preceding paper (6) on the method of determining the osmotic pressure of colloids, which criteria have been rigorously followed throughout the present series of observations, it is found that a much longer time is often required for the attainment of a true osmotic equilibrium in the case of lymph than in the case of serum. This delay may be attributed to the presence of fat, which tends to decrease the permeability of the membrane. The fat content of the lymph from most of my animals was not especially high, for they were fed on skimmed milk. But in a control experiment (described in the section on methods) and in several preliminary trials of the method of collection of lymph, the animals received cream, and the lymph contained a relatively large amount of fat, as was evidenced by the great difficulty encountered in the digestion of the sample with sulphuric acid in the determination of the nitrogen content. The determination of the osmotic pressure of such samples of lymph usually required more than 12 hours as compared with the usual time of 4 to 5 hours required for the attainment of equilibrium for lymph of low fat content, and 1 to 3 hours for serum. The suggestion which has been recently made (14) that lipids in a state of dispersion as they occur in serum during lipemia may in themselves be responsible for an osmotic effect was tested out, so far as the fat content of lymph is concerned, in the case of a sample with high fat content which was taken from the thoracic duct. The osmotic pressure per gram of protein was 44.4 mm. of water which is not higher than can be accounted for by the protein content of 4.07 per cent and the albumin: globulin ratio of 1.7. It is therefore extremely doubtful whether fat can exert an appreciable effect on the equilibrium osmotic pressure of colloids, even though it may undoubtedly delay the attainment of the true osmotic equilibrium.

It is well known that the initial pressure which is exerted by serum against an outer solution of salts is considerably higher than the equilibrium osmotic pressure. The excess of pressure is presumed to be due to non-protein substances which penetrate through the membrane with difficulty. Any condition, such as the presence of fat on the membrane, which delays the diffusion of these substances will prolong the period of excessive pressure. With slow membranes it is often exceedingly difficult to observe the gradual fall of pressure as these substances diffuse slowly into the outer solution. The process may require hours, days or weeks, depending on the permeability of the membrane, its thickness, etc. Readings of the osmotic pressure under such conditions are apt to be too high by a constant figure which will be proportional to the concentration of these slowly diffusing substances, other conditions being the same. Presumably the concentrations of these materials will be about the same in all similar samples of biological fluids from normal animals. Therefore the error in

the values of the colloid osmotic pressure which may appear as the result of neglecting the temporary pressures of these substances, will be a relatively constant error. The occurrence of a constant error in a series of values will produce a relatively large error in the calculated specific osmotic pressure for low protein contents and a relatively small error in the corresponding calculated values for high protein contents. In fact, it becomes possible to recalculate the figures obtained by the above-mentioned authors, allowing for a constant error, and thereby to obtain figures for the specific osmotic pressure which *increase* in a normal manner as the protein content of the lymph samples increases.

Whatever may be the reason for the great discrepancy between the osmotic pressure values for lymph which have previously been reported as compared with those presented in table 1, it is believed that the latter values are as nearly correct as can be obtained by a method in which rigid attention to details of the preparation and standardization of membranes and a close adherence to the necessary criteria for the estimation of the true equilibrium pressure have been practiced.

Total osmotic pressure. In the last column of table 1 are presented the figures for the total osmotic pressures of colloids of serum and of lymph. The bracketed figure was calculated from the total protein content of the lymph on the assumption that the specific osmotic pressure was 40 mm. water.

Relation between effective osmotic pressure and mesenteric venous pressure. Figure 1 presents in graphic form the relations found to exist between the "surplus" osmotic pressure of the blood (i.e., the osmotic pressure of colloids of serum *minus* the osmotic pressure of colloids of lymph) and the mesenteric venous pressure. It is probable that the surplus osmotic pressure is, in the case of the intestine, a fairly accurate measure of the "effective" osmotic pressure of the blood. This assumption is based on the fact that the flow of lymph from the intestine is continuous, at least during digestion. In the words of Krogh, Landis and Turner (15) "The more rapid the filtration . . . the more nearly will both average tissue fluid and lymph resemble the fluid filtered through the capillary wall." It therefore seems justifiable to assume that, on the average, the walls of the intestinal capillaries are permeable to the same fraction of the total concentration of plasma proteins as is found in the lymph from the lacteals, and that the surplus osmotic pressure is in reality of the same magnitude as the effective osmotic pressure. How then can we account for the fact that the effective osmotic pressure is as high as, or even higher than, the mesenteric venous pressure? Krogh (9) assumes that the mesenteric venous pressure is only slightly lower than the pressure in the intestinal capillaries. If this were true it would require a much lower effective osmotic pressure than has been calculated from the experiments of this series to allow a continuous

formation of lymph by filtration through these capillaries. Appropriate low figures for the effective osmotic pressure would require that the osmotic pressure of lymph be considerably higher than I have found it to be. The difficulty disappears, however, when we recall the recent observations of Landis (16) who found that the pressures in the capillaries of the mesentery of the rat and the guinea pig are considerably higher than the mesenteric venous pressure. For example, corresponding to a mesenteric pressure of 120 mm. water the arteriolar capillary pressure was 220 mm. and the venous capillary pressure was 150 mm., giving a mean

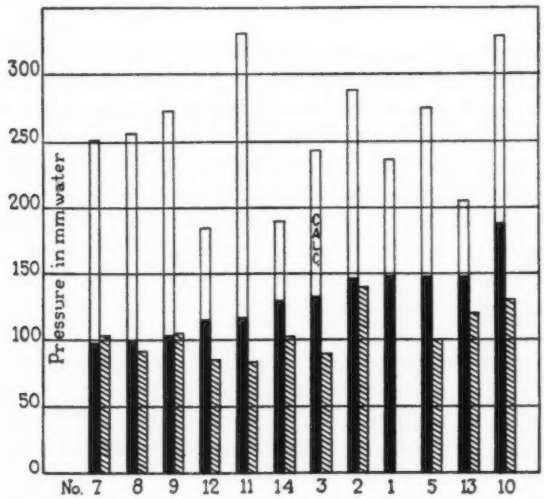

Fig. 1. Relation between the "surplus" osmotic pressure of serum and the mesenteric venous pressure. Data arranged by experiments in ascending order of the surplus osmotic pressures. Total height of black and white columns: Osmotic pressure of colloids of serum. Height of white columns: osmotic pressure of colloids of lymph. Height of black columns: difference between osmotic pressures of serum and lymph = surplus osmotic pressure of serum. Cross-hatched columns: lateral pressure in mesenteric vein.

capillary pressure of 185 mm. water. Although no measurements of the pressure have been carried out on the capillaries of the intestinal villi it is very probable that these pressures are considerably higher, perhaps 50 per cent higher, than the mesenteric venous pressures. If this is the case it becomes easy to understand how filtration may proceed continuously even when opposed by the relatively high effective osmotic pressures calculated from the data of these experiments.

Although the conclusion that lymph formation in the intestine occurs

as a result of capillary filtration appears to be valid, it cannot be definitely established until direct measurements of the capillary pressure in this region can be carried out.

SUMMARY

1. A method for the collection of intestinal lymph from the mesenteric lacteals of the dog is described.

2. The concentration and osmotic pressure of the proteins of this lymph as well as of blood serum were determined in a series of 12 dogs, and the mesenteric venous pressure was measured in the majority of the experiments.

3. The albumin:globulin ratio of the lymph is somewhat higher in most instances than the corresponding ratio for the serum, a finding which indicates that the capillaries of the intestine exhibit a slight degree of selective permeability with respect to these protein fractions.

4. The ratios of the total protein content of lymph: total protein of serum vary from 0.3 to nearly 0.7. It is suggested that the protein content of the lymph of any individual dog is determined, within these limits, by the protein content of the serum of the animal. The absolute variation in each case is proportional to the protein content of the serum so that for each 1 per cent of serum protein the lymph protein may show a variation of 0.4 per cent.

5. The specific osmotic pressure of proteins is slightly lower for lymph than for serum, the difference being due, presumably, to the lower concentration of protein of the lymph. The recent finding, by others, of a higher specific pressure for lymph from the thoracic duct and cervical region than for the serum of the same animal is discussed.

6. The mesenteric venous pressure is either of the same magnitude or slightly lower than the calculated effective osmotic pressure of the blood. In view of the recent determinations by Landis of the steep pressure gradient in the capillaries of the mammalian mesentery, it is believed that the average capillary pressure in the intestine is sufficiently higher than the mesenteric venous pressure to account for the continuous formation of lymph by a process of filtration of a protein-containing fluid from the capillaries of the region.

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THE PASSAGE OF MATERIALS THROUGH THE INTESTINAL WALL

II. The Osmotic Pressure of the Colloids of Lymph from the Lacteals as a Measure of the Absorbing Force of the Intestine

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Received for publication May 7, 1932

From the experiments (1) on the absorption of water from physiological saline solution placed in isolated jejunal loops of dogs it has been previously shown that the intestine exhibits a definite absorbing force, and that this force is accurately measured by the extent to which the intra-intestinal pressure must be lowered below atmospheric in order to prevent the absorption of fluid. It was suggested that the absorbing force may reside in the osmotic pressure of colloids of the tissue fluids of the villi, and it was urged that this hypothesis be tested by making a comparison, in individual animals, of the magnitude of the absorbing force with that of the osmotic pressure of the lymph from the lacteals.

From the data to be considered in the present communication it appears that the osmotic pressure of the proteins of the lacteal lymph is of the same magnitude as the absorbing force of the intestine. This conclusion is reached after consideration of data obtained from a series of 14 experiments on dogs in the course of which determinations were made of the osmotic pressure of colloids of blood serum and of lymph from the lacteals; of the total protein, albumin, and globulin content of lymph and of serum; of the mesenteric venous pressure; and of the absorbing force of the jejunum. The animals were anesthetized with barbital. With the exception of those findings which relate most directly to the topic under discussion, the data resulting from these experiments have been presented in detail in the preceding paper in This Journal (2) and have there been analyzed in relation to the question of the mechanism of lymph formation in the intestine.

It was realized from the beginning of the investigation that the validity of any conclusions which might be drawn from the comparison of the osmotic pressure of lymph with the absorbing force would rest, first of all on the absolute degree of accuracy of the measurement of these two factors,

and secondly on the validity of the assumption that the osmotic pressure of the lacteal lymph is approximately the same as that of the tissue fluids which bathe the inner surface of the epithelial membrane of the intestine. The absorbing force may be determined with a considerable degree of accuracy. Measurements of the osmotic pressure of colloids in biological fluids are, however, always subject to possibilities of error. It has therefore been necessary to develop a method for the measurement of the osmotic pressure and, no other standard being available, to compare the values obtained by this method with values obtained by other investigators. In order to make such a comparison it was essential to determine the concentration of total protein and of the albumin and globulin fractions of the fluids, for the osmotic pressure of colloids is determined mainly by these constituents. The necessity for carrying out the analyses was further indicated by the fact that Loewen and his colleagues in Drinker's laboratory (4), who are the only other investigators to report figures for colloid osmotic pressure of lymph, found values which present marked variations in the relation of the pressure to the protein content and are also much higher than would be expected from the total protein content of the samples. These authors suggested that the relatively high values were due to the presence in lymph of a relatively high concentration of albumin. The results of my studies, which have been presented in the two preceding papers, indicate that the irregular and relatively high values found by these authors cannot be explained on the basis of the protein analyses of lymph and therefore are probably not true equilibrium values. These studies have indicated further that the method employed in this investigation does give true equilibrium values, that these values bear the same general relation to the protein content that are in evidence for corresponding samples of serum, and that the values for blood serum obtained by the same method correspond very well with the most reliable figures for the colloid osmotic pressure of serum which have been published by workers in other laboratories. It is therefore believed that the method provides results which are sufficiently close to the absolute values to allow a comparison to be made of the osmotic pressure of lymph with the absorbing force.

As regards the identity of lacteal lymph and the tissue fluids of the villi, it must be admitted that our knowledge of the mechanism of formation of lymph is inadequate to answer the question in an entirely satisfactory manner. It may be presumed, however, that the rapid flow of lymph which occurs in the intestine especially during digestion will, in the first place, favor the attainment of equilibrium between the protein concentration of the lymph and that of the average tissue fluids and, in the second place, will tend to insure that the lymph, in its passage from the villi to the lacteals of the mesentery, will have undergone minimal changes in composition. In the preceding paper (2) the results of a comparison of

the mesenteric venous pressure with the calculated effective osmotic pressure of the blood lend further support to the assumption that the tissue fluids of the villi are being constantly renewed as a result of the continuous filtration which must occur in this region. It should be emphasized that, in the controversy which has recently arisen in regard to the possibility that the tissue fluids can contain protein in high concentration, the special case of the intestine has always been excepted, for it is generally agreed that the capillaries in this region are relatively more permeable to protein than the capillaries elsewhere in the body, with the possible exception of the liver.

METHODS. The experimental methods, with the exception of the procedure for the determination of the absorbing force, have been described in detail in the two preceding papers, the first of which (3) is given over entirely to the method for the measurement of the osmotic pressure of colloids.

The determination of the absorbing force of the intestine. The determination of the absorbing force was carried out immediately after the lymph had been collected. The same general method was employed as was described in the first paper of the series (1). However, in spite of the fact that it has been found for the case of fasting dogs that the absorbing force remains very nearly constant over long periods of time, it was thought that with the changes which might occur in the digestive state it would be advisable to make as rapid a determination as possible. Consequently no attempt was made to plot the absorption curves. The intra-intestinal pressure was lowered at once to a level which might be expected to nearly abolish absorption. By shifting the pressure above and below this point it was usually possible, in the course of from one to three hours, to find a point at which absorption could just be detected and also to find a second point at a somewhat lower pressure, where "negative absorption" would occur. In most of the experiments the absorbing force of the gut could be determined within a fairly narrow range of pressures. The extent of the range of pressures within which the critical pressure can be found to lie is dependent largely on the relative intensity of movements of the gut. Peristalsis was reduced to a minimum by maintaining the temperature of the room at 30°C. and by exposing the isolated loop to air saturated with water vapor at a temperature of 38°C. to 40°C. This was accomplished by the use of a box containing a temperature regulator and humidifying device, so adapted in shape that it could be placed over the abdomen of the animal.

The horizontal diameter of the lumen of the isolated loop was taken as the reference level for the measurement of the intra-intestinal pressure.

PRESENTATION OF DATA. Figure 1 presents in graphic form the results of all experiments performed to test the degree of correlation between the osmotic pressure of colloids of lymph from the lacteals and the absorbing

force of the jejunum. It will be noted that in eight out of the fourteen experiments, those numbered 13, 14, 12, 1, 10, 2, 7, and 9 in the order of arrangement, there is a relatively close correspondence between the values for these two factors. In two additional cases (experiments numbered 5 and 6) the correspondence is more apparent than real, for the upper limit of the absorbing force could not be determined, due to slight hemorrhage into the bowel. Experiment 8 presents results which, due to this same interfering factor, are of little value, except in so far as the close correspond-

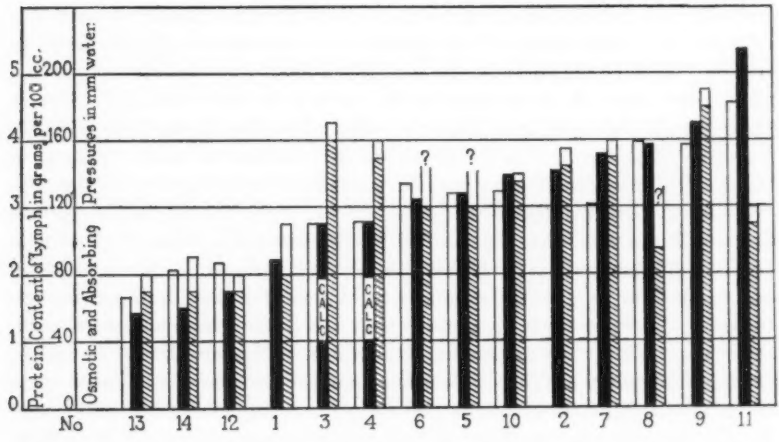

Fig. 1. Correlation of the osmotic pressure of colloids of lymph from the lacteals with the absorbing force of the intestine. The results are arranged in ascending order of the osmotic pressures of the lymph.

White columns: protein concentration of lymph in grams per 100 cc.

Solid black columns: Osmotic pressure of colloids of lymph in millimeters of water.

Cross-hatched columns with white tops: absorbing force of the gut in millimeters of water determined within the limits indicated by the heights above the base line of the base and top of the white area. The scale of protein concentration is so related to the scale of pressures that a one per cent protein content corresponds to an osmotic pressure of 40 mm. of water, which is not far removed from the average of all determinations. (See the previous paper (2) for the tabulated data.)

ence between the protein content and the osmotic pressure of the lymph is exemplified. In experiments 3 and 4 the osmotic pressures were not determined. The approximate values, calculated from the protein content of the lymph samples, are significantly lower than the corresponding values for the absorbing force of the gut. Experiment 11 presents, on the other hand, the single example in which the absorbing force is lower than the osmotic pressure of the lymph.

It should be pointed out that the occasional lack of agreement between

the osmotic pressure and the absorbing force might well be expected in view of the time (several hours) which necessarily elapsed between the collection of the lymph and the measurement of the absorbing force.[1] In this interval certain factors may occasionally operate to produce changes in the composition of the tissue fluids of the villi. In the first place a profuse (though usually transitory) secretion of intestinal juice occurs as the result of the mechanical stimulation due to the necessary manipulation of the gut. In the second place, the animal is in the digestive state and the whole intestinal tract is therefore in a condition of functional activity. By reason of circulatory or other changes associated with this activity the composition of the lymph may presumably change to some extent. It is obvious that the lymph taken from the lacteals of the mesentery may, in proportion to the extent of such changes, differ in composition from the lymph in the villi, which has been more recently formed. The fact that a rather wide range of protein concentrations of the lymph has been encountered in *different* animals has led to the conclusion (2) that variations can occur in the *same* animal within certain definite limits which are determined by the protein content of the serum of the individual animal. On the basis of such variations it becomes possible to account in a fairly satisfactory manner for the discrepancies, referred to above, between the osmotic pressure of the lymph and the absorbing force. Thus, in experiment 3 the ratio of the protein of lymph to protein of serum was 0.52. Assuming that previous to the determination of the absorbing force the ratio had risen to the upper limit of 0.64, there would have been an increase in the protein concentration of the lymph from 2.75 per cent to 3.4 per cent. The corresponding increase in the osmotic pressure would be sufficient to reduce the discrepancy between osmotic pressure and absorbing force by nearly 50 per cent. Similarly the discrepancies of experiments 4 and 11 can be accounted for *completely* without the necessity for assuming a relatively greater change in the protein content of the lymph for the individual animal than could be accounted for by the variations observed in the series of animals (2).

In the data presented in the first paper of this series (1) values for the absorbing force of the gut were found to lie between 80 mm. and 260 mm. of water. In subsequent experiments (unpublished) values as low as 40 mm. of water have occasionally been observed. In the series of experiments here reported the osmotic pressures of the lymph range from 57 mm. to 214 mm. of water. The failure to observe as high a value for the osmotic

[1] It has not seemed feasible to attempt to collect the lymph *after* the determination of the absorbing force, for, as the result of the operative procedures incident to the preparation of the isolated loop, the lymphatics of this loop as well as those of remaining parts of the intestine become constricted to such a degree that it would be very difficult to collect a sufficient amount of lymph from them.

pressure or for the absorbing force as was encountered in the first series of studies is doubtless to be attributed to the fact that the protein content of the sera of the dogs of this series was relatively low as compared to the normal range of protein concentrations for dog serum. Loewen (4) encountered a serum concentration of 8.84 per cent, with a corresponding value for thoracic duct lymph of 4.93 per cent. Allowing an osmotic pressure per one per cent protein of 47.8 mm. water (the highest value encountered for lymph in the present series) this lymph would have had an osmotic pressure of 236 mm. water. Meyer-Bisch and Günther (5) obtained from the thoracic duct of a normal dog lymph having a protein content of 7.2 per cent, with a correspondingly high value, 9.52 per cent, for the serum protein concentration. The osmotic pressure of this sample of lymph might well have been as high as 400 mm. of water. Although it is unlikely that the lacteal lymph will have a protein content as high as 7 per cent, under ordinary conditions, nevertheless it seems justifiable to assume that the absorbing force of the gut may vary within rather wider limits than have been encountered either in the present series of experiments or in the preceding investigations.

It has been pointed out (2) that the protein content of lymph from the lacteals appears to be fixed within definite limits by the protein content of the serum. Since the power of the intestine to absorb water resides in the osmotic pressure of the proteins of this lymph it follows that the absorbing force of the gut is fixed within definite limits by the protein content of the serum of the animal. This fact is related on the one hand to the general theory of the regulation of the water balance of the body and on the other hand it suggests certain possibilities in relation to the pathological physiology of some of the diseases in which the serum protein concentration is reduced below the normal level.

Discussion. The osmotic theory of absorption having survived the critical test represented by the direct comparison of the absorbing force of the gut with the osmotic pressure of colloids of the lymph, it will now be desirable, on the one hand, to present as clear a picture of the process of absorption of fluid as is possible with the information at our disposal, and, on the other hand, to demonstrate that the osmotic theory can account for many of the observations concerning the absorption of fluid for whose explanation it has previously been considered necessary to invoke an active intervention of a "vital" force.

According to the osmotic theory the absorbing force of the intestine is due to the osmotic pressure, exerted against the semi-permeable epithelial membrane of the intestine, of that fraction of the total protein concentration of the blood serum to which, on the average, the blood capillary wall is permeable. This fraction of the serum proteins will pass by diffusion into any fluid which may be present between the capillary wall and the

epithelial wall of the gut, and diffusion will not be abolished, however much it may be retarded, by a flow of fluid in the opposite direction from the tissue spaces into the capillaries. The fact, however, that lymph appears to be formed continuously in the intestinal wall argues against the occurrence of any appreciable amount of absorption of fluid by the capillaries of this region and speaks rather for a constant and effective renewal of the protein-containing fluid in the tissue spaces of the villi by filtration from the capillaries. If one visualizes such a flow of fluid from the capillaries into the tissues and thence into the lymphatics one sees that it will provide for a certain amount of stirring of the fluids in the tissue spaces, a factor which is of great importance in the dynamics of osmosis. Since this stirring can be effective only in case the moving stream of fluid comes into actual contact with the osmotic membrane, it is of interest to note that the capillaries of the villus really are in close proximity to the epithelial wall. This proximity has formerly been considered as favoring the absorption of materials into the blood vessels and as placing the capillaries in a distinctly advantageous position, in this respect, as compared to the more distantly located central lacteal. It would now appear that the location of the capillaries close to the epithelium may rather be related to the maintenance of the absorbing force at a constant energy level.

It should be pointed out that materials which are dissolved in the absorbed water may readily pass by diffusion into the capillaries. Such diffusion may be retarded by the outward flow of fluid from the capillaries, but as this flow is probably not very rapid it cannot be expected to prevent the attainment of an approximate concentration equilibrium between the diffusible constituents of the tissue fluids and those of the blood. Additional provision for the attainment of such an equilibrium is perhaps furnished by the interchange of substances between blood and lymph which may be presumed to occur, due to the close proximity of thin-walled venules and lymphatic vessels in the submucosa (6). The disposal of the droplets of neutral fat, which are said to be synthesized in the villi from the absorbed fatty acid and glycerol components, may be a very complicated process but it seems highly probable that the majority of these relatively large particles will pass more readily along with the fluid stream and will enter the lymphatics, whose membranous lining is relatively permeable in comparison with the endothelium of the blood capillaries.

The picture of the process of absorption and disposal of various materials which has been presented above will account for many of the known facts. It should be emphasized, however, that the question of the disposal of material after it has been absorbed will not be completely solved until further knowledge concerning the mechanism of the exchange of fluids between the capillaries, tissue spaces and lymphatics becomes available. Absorption, on the other hand, at least as it relates to passage of *fluid*

through the epithelial lining of the gut, appears to be accounted for completely by the osmotic theory.

In this connection it should be mentioned that Starling, who first suggested a theory of absorption based on the osmotic pressure of colloids (7), was unable to accept his own suggestion by reason of certain facts which he considered provided evidence of an active intervention of the epithelial cells in the absorption process. An analysis of these objections, which have recurred again and again in discussions of the problem of intestinal absorption, reveals the fact that they do not rest on any firm basis of experiment and that, in truth, many of the observations which have been adduced as proof of the existence of a "vital" absorbing force can be more easily explained by the osmotic theory.

It is true that absorption studies have disclosed some very puzzling facts in relation to the selective absorption of various dissolved substances. Although further work along these lines is necessary it may be tentatively assumed that selective absorption has to do with selective permeability relationships between the membrane in question and the various molecular and ionic species which are presented to this membrane. It does not seem to be necessary, at present, to assume that the selection involves an expenditure of energy. Neither does the question of the absorption of *fluid*, with which the osmotic theory of absorption is concerned, enter, except very indirectly, into the problem of the selective absorption of dissolved substances.

The apparent irreciprocal or one-way permeability of the intestine with respect to certain substances, notably glucose, has offered another obstacle to the acceptance of a mechanical theory of absorption, although a careful analysis of the data of experiments which have been carried out to settle the question of the existence of such a one-way permeability forces one to the conclusion that no adequate test of the matter has yet been applied. In the absence of a single crucial experiment it will be best to leave the matter as it stands, realizing that the passage of dissolved substances through the gut wall may possibly involve the intervention of factors which are not concerned directly with the absorption of water.

The fact that water may be absorbed from a solution of sodium chloride which is slightly hypertonic with respect to the blood serum has been considered by Heidenhain, Starling and others as irrefutable evidence against a physical theory of absorption. Starling (7) demonstrates that theoretically the colloids of the blood could cause the complete absorption of such a solution, but only after it has become diluted by a preliminary passage of fluid into the gut until the intestinal contents have become isotonic with the blood. The fact that no such preliminary period is necessary, as is shown by the fact that the absorption of fluid from such a solution commences at once, forced Starling to the conclusion that we have to deal with

some peculiar intervention on the part of the epithelial cells—with a mysterious energy-consuming process, by which water may be transferred against an osmotic gradient. That this is unnecessary is indicated by the experiments of Lazarus-Barlow (8) who has shown that fluid can pass by osmosis from a solution of higher osmolar concentration to a solution of lower osmolar concentration provided the latter solution contains molecules which are either relatively or absolutely non-diffusible through the osmotic membrane which separates the two solutions. For example, fluid was observed to pass into ox serum from solutions of sodium chloride varying, in different experiments, from 1.5 to 2.0 per cent. The solution was separated from the serum by a peritoneal membrane. The concentrations of sodium chloride from which fluid might be removed by such "negative" osmosis are slightly higher than the concentrations at which the similar phenomenon of immediate absorption of hypertonic solutions from the gut may occur. The explanation of this "anomalous" process is obviously the same in both cases: the energy of diffusion of the more diffusible substance provides the energy for the transfer of water, in the direction of diffusion, against the osmotic gradient. This phenomenon has been carefully studied and its thermodynamic relation discussed by Shreinemakers (9). It is unfortunate that this possibility for the transfer of fluid against a gradient of osmolar concentration has not been more generally realized by physiologists. The failure to take such processes into account has contributed to many of the erroneous statements about the possibilities of the transfer of fluids which have appeared in the standard texts. For the present purpose it is only necessary to point out that the presence in the tissue fluids of the villi of the non-diffusing protein molecules makes possible, within certain limits, the absorption of "hypertonic" solutions of freely diffusible materials.

Another observation which has been taken as evidence for the activity of the cells during absorption is that isotonic sodium chloride solution will pass through the wall of a surviving intestinal membrane only in the direction from mucosa towards the serosa (10), (11). In these experiments, however, the protein content of the tissue spaces can account for the transfer of any amount of fluid in the above-mentioned direction, for there is no effective barrier to the passage of protein into the solution which is on the side of the serosa. The lymphatics are known to be permeable to protein. The capillaries, after the circulation has been interrupted, are probably almost completely permeable to this material. (But even if they remain impermeable it makes no essential difference in the development of the argument.) No impermeable membrane being present in the tissues of the gut wall to prevent the outward diffusion of protein, the whole gut wall, with the exception of the epithelium of the mucosa, becomes, in effect, a spongy mass of tissue with protein solution enclosed within its meshes.

The ligation of the larger lymphatics and blood vessels will not alter the situation. The protein solution within this sponge becomes, in fact, a continuous part of the outer solution of saline, with this qualification, that the bulk of the protein is held in close contact with the semipermeable epithelial membrane. If one should soak a thick layer of filter paper in blood serum and place it against one side of a collodion membrane which is semipermeable with respect to protein, and should place a solution of sodium chloride on either side it would require no invocation of a vital force to explain the resulting osmosis. The same reasoning applies to the case of the absorption, in one direction, of fluid through the excised skin of the frog (12).

The only experimental fact which is at all difficult to reconcile with the osmotic theory of absorption is that blood serum may be absorbed from the intestine. It is commonly assumed that the serum, under these conditions, will continue to exert its full osmotic pressure of colloids, but most investigators are agreed that the total amount of protein of the serum decreases to some extent, even though the concentration may be increased by the absorption of fluid. If this is true it would indicate very clearly that the protein is being broken down, presumably by the action of some digestive enzyme. If such digestion occurs it probably takes place most actively near the epithelial cells, so that one may easily suppose that the osmotic activity of the layer of serum nearest the cells is thereby abolished. It is possible, also, that other types of changes may take place in the serum which will reduce its osmotic activity. In no instance has the experiment been correctly controlled by an actual determination of the osmotic pressure of colloids of the serum remaining in the gut after the period of absorption. There is the further possibility that the absorption of fluid from serum may in part be due to increased intra-intestinal pressure. A further study of this problem is distinctly indicated but it is to be doubted that the presence of serum in the intestine can unmask a hidden mechanism for the absorption of water which has given no hint of its existence in the course of the investigations here reported.

It might well be emphasized at this point that the osmotic theory provides for the complete absorption of all fluid from the intestinal tract, unless this fluid contains in solution substances the particles of which are entirely unable to pass through the intestinal membrane, and which in addition can continuously exert an osmotic pressure sufficient to counterbalance the absorbing force of the proteins of the tissue fluids. The transformation of colloidal food materials into diffusible forms as the result of the processes of digestion is therefore to be considered not merely as a necessary preliminary step in the absorption of the food itself but as a process which may facilitate, at the same time, the absorption of the watery contents of the intestinal tract.

With the tentative exception of the absorption of water from serum the osmotic theory meets the main objections which have formerly been considered as sufficient grounds for ruling out any mechanical theory of absorption. The theory does not, however, attempt to evade the necessity for the expenditure of energy. This energy does not appear to reside in the epithelial cells nor in any other part of the intestine, at least under normal conditions, but is to be considered as being derived from those processes of the body which have to do with the maintenance of the osmotic pressure of the colloids of the blood. In so far as the amount and osmotic properties of the blood proteins and the condition of the capillary circulation in the villi remain unchanged, then, the potential of osmotic energy available for absorption of fluid can be maintained only by the elimination of water from the body. From a philosophical viewpoint, therefore, the "vital force," the Maxwellian demon (12), which has been invoked in the past to explain the intestinal absorption of fluid, is, as a consequence of these deductions from the osmotic theory, dispossessed from its residence in the enteric epithelium and exiled to other localities. The responsibility for the final eviction of this pest rests, henceforth, on those who are engaged in the study of the mechanisms by which water is excreted.

The elimination of the element of mystery from the absorption problem should encourage the further study of the regulatory mechanisms which, under normal and pathological conditions, are able to affect the direction and rate of transfer of water through the intestinal membrane.

SUMMARY

1. The osmotic pressure of colloids of lymph from the lacteals is found to correspond closely to the absorbing force of the intestine as measured by the negative intra-intestinal pressure necessary to abolish the absorption of fluid from isolated jejunal loops of dogs.

2. It is shown that, in general, the absorbing force is proportional to the protein concentration of the blood of the individual animal.

3. The findings indicate that the absorbing force of the intestine is due to the osmotic pressure, exerted against the semipermeable epithelial membrane of the intestine, of that fraction of the total serum proteins to which, on the average, the walls of the blood capillaries of the villus are permeable.

4. The probable relations of the process of formation of lymph in the intestine to the processes of absorption and disposal of fluid are considered.

5. The osmotic theory of absorption is subjected to the test of meeting the objections which have so often been raised against the acceptance of a physical theory of absorption of fluids.

6. From the osmotic theory it may be deduced that the energy potential of the absorbing force is maintained by those processes of the body which

govern the concentration of the blood proteins. In the normal animal these forces are remote from the intestine and are to be found in the organs of the body which have to do with the excretion of water.

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THE RESPIRATORY QUOTIENT OF THE BRAIN[1]

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Received for publication May 7, 1932

Although the respiratory quotient of the entire organism has been the subject of many investigations, the respiratory quotient of individual organs has not received the same exhaustive attention. However, the respiratory quotient has been determined for muscle (Himwich and Castle, 1927; Himwich and Rose, 1929; Doisy and Beckmann, 1922; Hines, Leese and Knowlton, 1931; Takane, 1926; Richardson, Shorr and Loebel, 1930); testicle (Himwich and Nahum, 1929; Shorr, Loebel and Richardson, 1930; Dickens and Simer, 1930, 1931); kidney (Richardson, Shorr, and Loebel, 1930; Dickens and Simer, 1930, 1931); and peripheral nerve (Fenn, 1927; Gerard and Meyerhof, 1927; Meyerhof and Schmitt, 1929). Loebel (1927) determined the respiratory quotient of cerebral tissue suspended in various nutritive media. A respiratory quotient of the brain in the living animal fixed at unity was reported by Himwich and Nahum (1929). The same value was obtained for excised brain tissue by Dickens and Simer (1930, 1931). Lennox (1931) working on human subjects obtained an average respiratory quotient of the brain of 0.95. The present paper contains the completed report of the respiratory quotient of brain observed in unanesthetized and amytalized dogs. The respiratory quotient was also determined after pancreatectomy, the injection of phlorhizin and various endocrine products; insulin, adrenalin, pituitrin, and pitressin.

METHOD. The brain of twenty amytalized dogs was studied after exposure of the superior longitudinal sinus and femoral artery so that blood samples could be drawn from both vessels practically simultaneously. Five unanesthetized animals: one normal, one injected with pituitrin and three with pitressin, were prepared under ether anesthesia a day or two previously to that on which the observations were made. Depancreatized dogs were usually examined 72 hours post-operative, the phlorhizinized animals after the D/N ratio had become constant. One animal was phlorhizinized after pancreatectomy. The endocrine extracts were injected subcutaneously and sufficient time was allowed to permit the full development of their actions before the blood samples were drawn.

[1] The expenses of this research were met in part by a grant from the Research Fund of the School of Medicine, Yale University.

The respiratory quotient of the brain was determined by analysis of samples of cerebral blood for carbon dioxide and oxygen content by the method of Van Slyke and Neill (1924). The carbon dioxide and oxygen capacity were also obtained after the blood had been exposed to a mixture of 5.5 per cent carbon dioxide in oxygen as described in previous reports (Himwich and Rose, 1929). Check determinations differed by 0.2 volume per cent or less. Previous to the withdrawal of the blood samples, expired air was collected and the respiratory quotient of the whole animal was determined by analysis of the expired air for carbon dioxide and oxygen with the Haldane-Henderson apparatus.

RESULTS. Table 1 contains a summary of the respiratory quotients of the normal, phlorhizinized, and depancreatized dogs, and table 2 illustrates the results of individual experiments. It may be seen that the respiratory quotients closely approximate 1 in each observation and that the average of 38 observations is unity. The average deviation of any respiratory quotient is $\pm$ 0.035 and the deviation of the mean is $\pm$0.006. The average respiratory quotient of three observations of the brain of two dogs injected with insulin is 1.01 $\pm$0.03. Two observations made on each of three dogs after injection of pitressin disclosed that in two dogs the respiratory quotients were not within the physiological range, probably the result of causes to be considered in the discussion. In the third animal, however, the respiratory quotients are 0.99 and 1.01. The brain of one dog receiving pituitrin has respiratory quotients of 1.08 and 1.02. Adrenalin like the pressor fraction of pituitrin affects the respiratory quotient, since in 7 of 10 observations of five dogs the respiratory quotient differed widely from unity.

In fifteen of eighteen observations the carbon dioxide capacity of the venous blood is greater than that of the arterial blood. The average increase is 0.74 vol. per cent. The average differences of the concentrations of the arterial and the venous blood are not significant. On eight occasions the venous blood is more concentrated disclosing an average increase of 0.34 vol. per cent and in nine other observations the venous blood is diluted on the average to the extent of 0.25 vol. per cent.

DISCUSSION. *Results.* That the respiratory quotient of the brain is unity has been substantiated by the results of Dickens and Simer (1930, 1931), obtained on brain tissue. The value of 0.95 observed on human brain by Lennox (1931) is in close agreement when one considers that this investigator obtained venous blood from the internal jugular vein which contains an admixture of blood from the brain and other parts of the head.

Foodstuffs utilized by the brain. The respiratory quotient of unity does not necessarily indicate that only carbohydrate is oxidized by the brain. It has been observed by Quastel[2] that glutamic acid is oxidized by excised

[2] Personal communication.

cerebral tissue. The respiratory quotient should be unity if the nitrogen atom forms urea. It is also possible that the oxidation of a combination of substances may yield a respiratory quotient of unity. For example, the respiratory quotient of succinic acid is 1.14 while that of protein without the formation of urea is 0.95. Moreover, succinic acid is readily oxidized by the brain (Quastel and Wheatley, 1931; Ashford and Holmes, 1931).

TABLE 1

NUMBER OF ANIMALS	CONDITION OF ANIMAL	NUMBER OF OBSERVATIONS	AVERAGE R. Q.
4	Normal	7	0.99
1	No anesthesia	4	1.04
4	Phlorhizinized	12	1.00
4	Depancreatized	11	0.99
1	Depancreatized and phlorhizinized	4	0.99
14		38	1.00±0.006

TABLE 2

Respiratory quotients of brain

CONDITION OF ANIMAL	AIR R. Q. OF INTACT ANIMAL	BLOOD R. Q. OF BRAIN
Fasted 2 days	0.75	1.03 1.01
Fasted 2 days; no anesthetic	0.77	1.06 0.97
Phlorhizinized	0.69	0.97 1.02
Depancreatized	0.67	0.94 1.00
Depancreatized and phlorhizinized	0.69	0.96 1.00 1.00 0.99

Richardson (1929) presents a list of various substances which have respiratory quotients close to unity. However, most of these substances, unlike glucose, appear in the blood in small concentrations. It is, therefore, probable that carbohydrate is the chief fuel of the brain.

In the normal animal (McGinty, 1929; Himwich and Nahum, 1929; and Nahum, Himwich and Koskoff, 1932) both glucose and lactic acid were

TABLE 3

Carbon dioxide and oxygen capacities of cerebral blood

DATE	KIND	CONDITION	CARBON DIOXIDE	OXYGEN
October 4, 1928	Arterial	Amytal	39.14	20.95
	Venous	Amytal	40.43	20.72
	Arterial	Amytal	40.15	21.18
	Venous	Amytal	39.78	21.28
November 1, 1928	Arterial	Amytal	40.61	14.82
	Venous	Amytal	41.83	15.46
	Arterial	Amytal	41.87	13.76
	Venous	Amytal	41.56	13.60
November 9, 1928	Arterial	No anesthesia	41.00	21.54
	Venous	No anesthesia	41.50	21.06
	Arterial	No anesthesia	41.22	
	Venous	No anesthesia	42.24	
November 10, 1928	Arterial	No anesthesia	42.00	19.47
	Venous	No anesthesia	42.94	19.40
	Arterial	No anesthesia	41.04	19.84
	Venous	No anesthesia	41.72	19.20
December 21, 1928	Arterial	Phlorhizin	37.07	20.94
	Venous	Phlorhizin	37.43	21.03
	Arterial	Phlorhizin	35.33	20.71
	Venous	Phlorhizin	36.34	21.00
November 15, 1928	Arterial	Depancreatized	41.40	22.31
	Venous	Depancreatized	41.79	22.63
February 14, 1929	Arterial	Depancreatized	37.69	18.50
	Venous	Depancreatized	37.51	18.63
	Arterial	Depancreatized	38.54	18.76
	Venous	Depancreatized	38.88	18.49
July 29, 1930	Arterial	Adrenalin	26.72	23.40
	Venous	Adrenalin	27.71	24.47
October 14, 1930	Arterial	Adrenalin	34.86	23.85
	Venous	Adrenalin	35.26	23.92
	Arterial	Adrenalin	32.01	24.07
	Venous	Adrenalin	32.14	23.92
January 11, 1932	Arterial	Pitressin	33.83	24.57
	Venous	Pitressin	34.88	24.37
	Arterial	Pitressin	31.90	23.84
	Venous	Pitressin	32.65	23.61

consistently absorbed from the blood by the brain. These substances are not changed to glycogen by the brain (Holmes and Ashford, 1930) nor does that organ store glycogen (Takahashi and Asher, 1925). According to Holmes (1930) the retained carbohydrate is oxidized by the brain but only after conversion to lactic acid for Holmes has demonstrated that cerebral tissue can utilize glucose only after transformation to lactic acid.

Glucose and lactic acid are removed from the blood by the brain of a diabetic animal (Himwich and Nahum, 1929; Nahum, Himwich and Koskoff, 1932). This glucose, however, cannot be oxidized directly for the respiratory quotient remains one even after pancreatectomy when the brain loses the insulin (Nothmann, 1925) necessary for the oxidation of glucose. It is therefore probable that the brain of the diabetic animal may also transform glucose to lactic acid before oxidation. Insulin is not required for the oxidation of lactic acid. Holmes and Holmes (1924) have found that the brain tissue of depancreatized cats oxidizes lactic acid.

It is evident that the respiratory quotient of the brain is not altered by insulin or pituitrin which are known to influence the character of the metabolism of the body as a whole. Adrenalin and pitressin, however, caused variations in the respiratory quotient of the brain not necessarily because of a change in the character of the foodstuffs oxidized but rather due to alterations in the acid-base, electrolyte and water equilibria. The acid-base equilibrium is shifted because of the production of increased amounts of lactic acid after injection of adrenalin (Tolstoi, Loebel, Levine and Richardson, 1924; Nahum and Himwich, 1931) or pitressin (Himwich and Fazikas, 1930; Bischoff and Long, 1931; Himwich, Haynes and Fazikas, 1932). In addition, adrenalin (Ederer, 1927; Haldi, Larkin and Wright, 1926) changes water and salt balances between tissues and blood, and pitressin probably is largely responsible for the effects of pituitrin on water and electrolyte (Underhill and Pack, 1923; Bushke, 1928) exchanges of the body.

The rise in the carbon dioxide capacity of the venous cerebral blood occurs despite the lower carbon dioxide capacity of the brain (Kleinschmidt, 1929) and is probably related to the constant removal of lactic acid from the blood passing through the brain. It is interesting to note that no change in the concentration of the cerebral blood is disclosed, probably due to the rapidity of the blood flow.

Method. Rapport (1930) has criticized the respiratory quotients of resting (Himwich and Castle, 1927) and exercising (Himwich and Rose, 1929) muscle obtained by the methods used in the present research because of possible sources of error due to differences in blood flow. Even admitting the possibility of sudden changes in blood flow during the withdrawal of the blood samples, the ratio of carbon dioxide produced and oxygen consumed would still be determined by the proportions of fat and carbo-

hydrate oxidized. The factors determining the accuracy of the method have been discussed previously (Himwich and Rose, 1929). They include an adequate gaseous exchange and the avoidance of changes in the acid-base and osmotic equilibria. In the present series of experiments these equilibria were disturbed only after the injection of adrenalin or pitressin. The gaseous exchange though not so large as in the experiments on exercising muscle was sufficiently great, approximately 10 vol. per cent both for carbon dioxide and oxygen and the average deviation of any quotient was ±0.035. Thus we see that by the observance of the proper experimental precautions each single respiratory quotient may be significant and an examination of table 2 reveals that most quotients are close to unity.

Oxygen consumption of the brain. With a cerebral blood flow of 140 cc. per 100 grams of tissue per minute (Jensen, 1904) and a removal of oxygen of 10 cc. per 100 cc. of blood (table 4), it may be calculated that the oxygen consumption is 14 cc. per 100 grams of brain per minute. This is much less than the oxygen used by the brain of smaller species for Holmes (1930)

TABLE 4

The average oxygen consumption of the brain and the entire organism

PREPARATION	NUMBER OF OBSERVATIONS	O_2 REMOVED PER 100 CC. OF BLOOD	O_2 CONSUMPTION OF ENTIRE ORGAN	AVERAGE WEIGHT OF DOG
Amytalized and unanesthetized	11	10.99	83.38	15.64
Phlorhizinized	12	9.99	98.72	16.34
Depancreatized	11	9.40	113.8	17.33

found a value of 20 cc. per 100 grams of rabbit per minute. It is logical to suppose that the oxygen consumption of human brain may be less than that of the dog.

Alexander and Cserna (1912) reported an oxygen consumption of 36 cc. per 100 grams of brain of dog per minute. However, on recalculation of their results the oxygen consumption was found to be 13 cc. per 100 grams per minute, a value which is in close agreement with the present observations. Schmidt (1928) perfused the brain of dogs under various conditions and obtained results of the same magnitude.

The average brain weight of 70 grams in our dogs indicates a blood flow of approximately 100 cc. per minute. It can be seen from table 4 that the brain consumes about 13 per cent, 8 per cent, and 10 per cent of the entire oxygen intake of the body of normal, depancreatized and phlorhizinized dogs respectively. The average of the three values is approximately 10 per cent of the total oxygen consumption. Taking the average value it may be calculated that a respiratory quotient of 0.70 obtained from the expired air indicates that the average respiratory quotient of the body,

exclusive of the brain, is 0.67, while the respiratory quotients between 0.70 and 1.00 are diminished proportionately, 0.80 to 0.78 and 0.90 to 0.89.

SUMMARY

The respiratory quotient of the brain of 14 dogs (1 unanesthetized, 4 amytalized, 4 phlorhizinized, 4 depancreatized and 1 depancreatized and phlorhizinized) has been determined by analysis of the arterial and venous blood for carbon dioxide and oxygen by the method of Van Slyke and Neill. The average of the 38 respiratory quotients thus obtained was 1.00 $\pm$0.006 and the average deviation of a single result was $\pm$0.035.

Four unanesthetized animals were studied after injection of pituitrin or pitressin and 7 dogs under amytal anesthesia were examined after injections of insulin or adrenalin. The respiratory quotient was not changed by insulin or pituitrin although it was by adrenalin and pitressin probably because of shifts in the water, electrolyte and acid-base equilibria.

Carbohydrate is constantly oxidized by the brain of the living animal. Since the respiratory quotient remains unity after pancreatectomy carbohydrate is not utilized in the form of glucose.

It has been calculated that the oxygen consumption of dog brain is 14 cc. per 100 grams of tissue per minute.

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STUDIES ON MAGNESIUM DEFICIENCY IN ANIMALS

II. Species Variation in Symptomatology of Magnesium Deprivation

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Received for publication May 9, 1932

When young rats are restricted to a ration containing only 1.8 parts per million of magnesium but adequate amounts of other dietary substances, they develop a characteristic and striking symptomatology that presages an early death. As was recently described in some detail (1), the animals pass successively through stages of vasodilatation, hyperirritability of the nervous system, cardiac arrhythmia, and fatal tonic-clonic convulsions. Furthermore, it was demonstrated at that time that magnesium deficiency per se is responsible for the syndrome; anorexia, inanition, or some unknown deficiency may be excluded from consideration. The evidence, in all, led to the conclusion that in at least one species magnesium is an essential element for normal functioning, growth and life.

As the symptomatology unfolded it became a matter of some interest to determine whether magnesium is indispensable to another species besides that represented by the rat. To that extent the necessity of the element for animal economy would be the more convincingly demonstrated. Further, by reference to the rat's behavior on a low magnesium diet as a standard it appeared important to ascertain whether lack of magnesium would be so severely manifested and whether the symptomatic pattern would be faithfully duplicated in another animal. Finally, it was recognized that the details of the pathogenesis of magnesium deficiency would be the more readily obtained from an animal more suited to metabolic studies than is the rat. Therefore dogs, representing a species seemingly appropriate for the purposes in mind, were restricted to the same diet as had been the rats. The ration was adequate in all respects except magnesium, of which it contained only 1.8 parts per million.

When young dogs, 6 to 7 weeks old and weighing 2.7 to 3 kgm., are limited to the magnesium-deficient diet, they exhibit like the rat a remarkable succession of symptoms which result always in death. Within two weeks the nail-beds, particularly of the forepaws, take on a flush and then

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in turn the tongue, eyelids, and buccal mucosa undergo the same change. By four to six weeks the ears become involved and the vasodilatation is complete; the distended vessels impart a florid appearance specifically to these skin areas. Then the vasodilatation gradually but entirely disappears, sometimes to recur at various intervals at a later time. The rubescence, the heightened color of the tongue and nail-beds from vascular engorgement, might suggest the presence of polycythemia vera. But there the correspondence ceases. The blood findings and course of the disorder are not indicative of polycythemia; for there is no erythrocytosis and the vasodilatation, after a comparatively short duration, is superseded by more baleful symptoms.

An extraordinary alteration occurs in the appearance of the extremities. The phalanges begin to spread apart, and the extremity from the region of the proximal end of the metatarsals downward increases greatly in size (fig. 1). The animal, instead of walking on its paws, bears much of its weight on its metatarsals; thereby the line of curvature of the nails, instead of projecting downward, arches out horizontally. Later the nails become so brittle that they break. These changes are particularly prominent in older animals that have survived on the diet for some length of time.

Loss of hair occurs generally over the body but is especially severe around the eyes, on the legs, tail, and abdomen, where small circumscribed areas may become completely denuded. Elsewhere what hair remains is dry and shaggy. The skin in the denuded areas of the abdomen, tail, and extremities undergoes erythematous changes, then desquamation, and may even go on to ulceration. Eventually the discharging ulcers may become dry and disappear, but usually fresh lesions appear nearby. All over the body the skin is dry and scurfy.

The ears, which were swollen and spongy during the hyperemic stage, become thick, leathery, and mottled; they even show old hematomata as evidence of previous hemorrhage. Lacrimal secretion may become persistent. The urine is deeply pigmented, but is normal in volume and free from blood.

After a month on the magnesium-deficient diet many but not all animals show evidences of increased irritability of the nervous system. That hyperexcitability does actually occur in some, if not all, cannot be doubted, because stimuli ignored by normal control animals are capable of releasing convulsions in the experimental group. For example, rattling of windows in a severe windstorm brought on seizures in several animals on the magnesium-poor ration, while control animals did not even manifest concern. Because the extent to which the irritability is heightened before rendering the experimental animals susceptible to convulsions is not always readily apparent, because the action of a stimulus cannot be predetermined, and

sometimes an exciting cause cannot even be discovered, and because abruptness and unexpectedness characterize the convulsive state, it became desirable to detect some sign which would infallibly indicate when the animal had reached the critical point in sensitivity. This objective was not attained. For a time we felt that rectal temperature would serve as a premonitory sign of impending convulsions; for in many instances a sharp drop in temperature was not uncommon several hours before a seizure.

Fig. 1. An animal, which had been deprived of magnesium for a considerable period of time, is shown with greatly swollen paws, which are much flattened, with widespread digits, and with horizontally arched nails.

This phenomenon, however, was not sufficiently constant to be reliable. Empirically we have found that if the dogs are started at an age of six weeks and a weight of 2.7 to 3 kgm., they usually have the first convulsion within five to seven weeks on the diet. Very often the animals have lost a slight amount of weight before the attack appears.

The pattern of the convulsions is remarkable for its constancy. The attack is ushered in without a cry. The susceptible animal, because of a disturbing noise or for no apparent reason, becomes restless and uneasy; it paces about the cage until it collapses on its side in convulsions. The

entire body, rigid from a tonic contraction, is thrown into a characteristic posture. The head is drawn back and the extremities are stiffly extended. The tongue, protruding slightly, may be perforated by clenched teeth. The skin is cyanotic. Respiration ceases during the attack and returns with muscular relaxation.

After a momentary subsidence of the tonic convulsion, tremors appear over the body and then clonic convulsions set in with the animal alternately flexing and extending its extremities. In flexion the fore extremities are drawn closely to the thorax with bending at the metacarpophalangeal joint. The eyeballs fluctuate in size. Respiration is rapid and shallow. This phase of the convulsive attack is not invariable but is usually seen.

After the tonic-clonic convulsions comes a period of exhaustion. From extreme muscular weakness the animal lies on its side, rarely attempting to rise. Lacrimal secretion drains from dull, shrunken eyes. There is discharge of nasal secretion, champing of the jaws, and drooling of regurgitated stomach contents from the mouth.

Usually the convulsive attacks follow one another serially with only brief intermissions during which the animal recovers from exhaustion. In one of these intervals the respiration, which had momentarily ceased during the phase of tonic contraction, is slowly returning to normal when it suddenly stops. After a short period of beating the heart likewise ceases. The animals always die during an exhaustion period when recovery is apparently in sight. In the initial attack the mortality is as high for dogs as it is for rats; 13 of 15 dogs, representing 86 per cent, succumbed during a recovery phase of the first series of convulsions.

In addition to the spectacular symptomatology which carries with it such a fatal outcome, the animals on the low magnesium diet show an interesting growth record (chart 1). The usual curve is one either of maintenance of weight, or slight decline; ordinarily a convulsive seizure takes off the animal before the loss is severe.

The degree of irritability of the nervous system, the weight record, and the length of the survival period depend largely on the extent to which the diet is deficient in magnesium, and the age and weight of the animal. Containing only 1.8 parts per million of magnesium, the diet which we employed is almost free from the element, so that the effects of its deficiency may be easily elicited. If it is desired, therefore, to produce the acute form of the disorder, the age and weight of the dogs become a most important consideration. In general, if animals are carefully selected within the age and weight range of 6 to 7 weeks and 2.7 to 3 kilograms respectively, the hyperexcitability is quite pronounced, the growth curve shows little rise, and the life span is short. If this detail is not minded and older dogs of heavier weight are placed on the diet, the deficiency is

always ultimately felt, but the growth record may be one of slight gain for a time and the survival period may be much prolonged.

It is certain that the foregoing symptomatology and growth record are due to a deficiency in magnesium, and not to inanition or the lack of an unknown substance. The animals on the magnesium-deficient ration give no evidence of diminution in food intake at any time. Further, the growth curves of control animals fed the magnesium-low diet plus 0.05373 per cent magnesium, added in the form of $MgSO_4 \cdot 7H_2O$, as contrasted with the records of the experimental animals on the magnesium-poor diet, leave

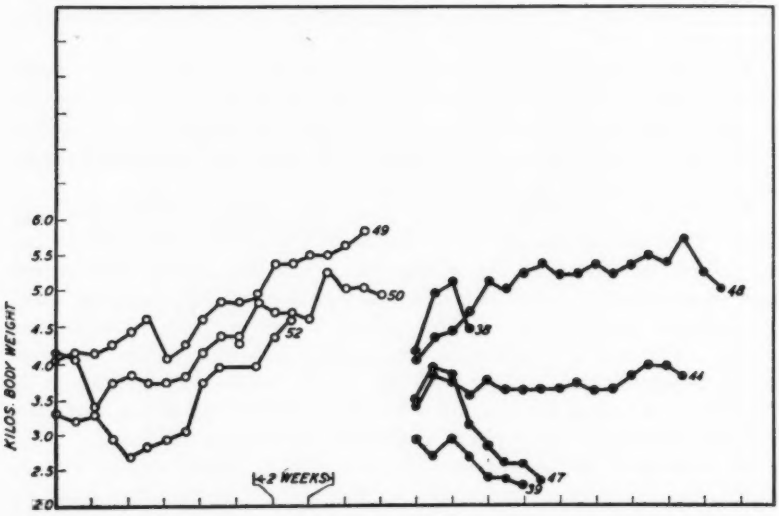

Chart 1. Weight curves of dogs. The curves with clear circles represent the weight records of dogs fed the magnesium-low diet plus added $MgSO_4 \cdot 7H_2O$. The curves with the solid circles represent the weight records of dogs fed the magnesium-low diet throughout life.* These latter curves likewise show the survival periods of the animals; the final circle representing the weight just before a fatal convulsion.

no doubt that the latter suffered from a true magnesium deficiency (chart 1). The control animals, given the magnesium-low diet with added magnesium from the start, showed gain in weight and freedom from symptoms for eighteen weeks, at the end of which time the experiment was terminated.

TECHNIQUE. For production of an exceedingly acute deficiency, that gives a short survival period with reasonably consistent results, it is essential that dogs be selected with an initial weight of 2.7 to 3 kgm., at an age of 6 to 7 weeks. If older dogs are inadvertently used, in time the mag-

* throughout the experimental period.

nesium deficiency will become just as manifest as in a younger dog, but the survival period is protracted.

In our experiments the dogs, although they showed no tendency to coprophagy, were confined in cages with screen bottoms. The animals had constant access to the diet and distilled water. The preparation of the magnesium-low diet, containing only 1.8 parts per million of the element, has already been described (1). For use with dogs we found it advisable to moisten the ration so that it could not be so readily scattered from the feeding pan. The control animals were given the magnesium-deficient ration plus 0.05373 per cent magnesium, added in the form of $MgSO_4 \cdot 7H_2O$.

Discussion. It will be recalled that we were interested in determining whether magnesium was indispensable to the dog, whether a deficiency in this component would be felt so severely, and whether the symptoms arising would follow substantially the same pattern as in the rat. That magnesium is essential to the dog is readily evidenced by the physiological disturbances, interruption of growth, and early death in the animals deprived of the element. By so much it becomes evident that magnesium is a nutrient necessary for animal economy.

Practically all the symptoms seen in the rat as the result of magnesium deprivation are likewise present in the dog consuming the same diet, so that qualitatively the deficiency is manifested in the same way in both species. Both show vasodilatation, hyperirritability, trophic disturbances, and generalized convulsions. Whatever variations appear between the symptomatology of the two species are slight, and are a matter of differences in intensity, time of appearance, and deviation. These slight differences are not surprising when the constitutional dissimilarities between the two species are taken into account. Furthermore, it must be remembered that the rats were offspring of a carefully controlled and uniform breed; whereas the dogs were of various breeds, and often the age was a matter of doubt.

The shades of intensity in symptomatology between the two species might well be mentioned in detail. In the dog, the vasodilatation does not stand out with quite the same prominence as in the rat, it is less intense and less generally distributed; the hyperirritability, while present in the dog, is not so apparent as in the rat; and the convulsions are less violent in the dog. The rat races at full speed just before going into convulsions, and his clonic contractions are much more vigorous and violent. On the other hand, the dog suffers much more in general nutrition and weight prior to the onset of convulsions than does the rat.

It is apparent that all these points deal with differences of degree such as would be expected from unlike species. We believe that one explanation is sufficient for all. Just as most other nutritional disorders give rise not only to constitutional effects, such as impaired nutrition, but also

local effects upon some system, so magnesium deficiency exerts an influence generally upon growth and weight, and locally upon the irritability of the nervous system. In humans there is a wide difference in the state of sensitivity of the nervous system in infants and adults; the nervous system of the former is inherently in a more irritable condition. It is not unreasonable to presume that such differences in sensitivity may arise from variations not only in age but also in species. Although no mensurable terms have ever been applied to contrast the nervous irritability of the dog with that of the rat, it is certain that differences must exist—by nature the rat is much more wild and excitable than the dog.

Accordingly, the normally irritable nervous system of the rat would suffer before the general effects of malnutrition would be felt. As a matter of fact, the young rats show vasodilatation and hyperexcitability, and die before nutritive loss sets in. On the other hand, the more stable nervous system of the dog does not fall under the localizing influence of the magnesium deprivation until the deficiency has made itself felt in malnutrition and loss of weight. Even here the general nutritive effect does not proceed far before increased irritability of nervous system leads to fatal convulsions. Actually then the results of magnesium deficiency are the same for the dog as for the rat, a general effect on nutrition and a local effect on the nervous system; but the relative prominence and order of appearance of the effects depend upon the inherent irritability of the nervous system in the two species. Whatever the slight differences in sensitivity between the two animals, it should not be forgotten that the influence on the nervous system is always felt and is always the cause of death in both.

Much the same reasoning may be applied in explaining why adult animals, both rats and dogs, show much more nutritive and trophic change before the onset of fatal convulsions, have their seizures at a later date, and survive so much longer than do young animals on the same magnesium-deficient diet. It may be that the greater magnesium store in the older animals enables them to withstand the deficiency for a longer time so that the tissues suffer in maintaining the magnesium of the blood at a normal level; that condition would explain the prominence of constitutional and trophic changes before the belated appearance of convulsions. It would seem more probable, however, that the greater susceptibility of the young animals to convulsions, which take them off before any striking nutritive changes occur, depends upon an inherently intensified irritability of the nervous system which is heightened the more by magnesium deficiency. By the same token, the nervous system of the adult animal possesses sufficient stability so that the whole body suffers from lack of magnesium as soon as or before the nervous system is sufficiently sensitive to respond with convulsions.

The dog, like the rat, manifests the effects of magnesium deficiency by the following symptom-complex: vasodilatation, hyperexcitability, and generalized convulsions. Regarded separately these symptoms might point to several conditions, but taken in association they are, we believe, indicative of tetany. In a previous publication (1) we stated at some length our reasons for viewing the syndrome as tetany. At that time we suggested that low-magnesium tetany was a distinct disorder, that vasodilatation was a vasomotor spasm, and that it was a characteristic feature differentiating low magnesium tetany from other forms, particularly the so-called infantile tetany. However, vasodilatation is not the only point of distinction between them. In the low-magnesium animals the absence of both laryngospasm and carpopedal spasm, which are so common in infantile tetany, is not without significance. Further study devoted to chemical changes in the blood and urine following magnesium deprivation may indicate additional points of difference.

SUMMARY

As in the rat, restriction of the dog to a diet containing only 1.8 parts per million of magnesium but adequate amounts of other dietary essentials leads to a symptom-complex of vasodilatation, hyperirritability of the nervous system, generalized convulsions, trophic changes, and nutritive failure. Although both species show all these symptoms, some are more prominent in the one than in the other. It is suggested that magnesium deficiency manifests itself generally by trophic disturbances and nutritive failure, and locally by increased irritability of the nervous system. In the dog the vasodilatation, hyperexcitability and convulsions are less intense while the trophic and nutritive changes are relatively more conspicuous than in the rat, because inherently the nervous system of the dog is more stable. In any event these differences are only a matter of degree because convulsions lead to death in both species. It is reiterated that the symptom-complex ensuing from a magnesium deficiency is tetany, differentiated from other forms principally but not solely by the vasomotor spasm.

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GLYCOGENOLYTIC EFFECT OF EPINEPHRINE ON SKELETAL MUSCLE

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Received for publication May 12, 1932

The conflicting data concerning the effect of epinephrine on glycogen in muscles may be accounted for by the fact that various investigators have removed the specimens of muscle under the influence of different anesthetic agents, or because different amounts of the substance have been injected into the body by different routes. Our investigations were undertaken to determine the effect of epinephrine on glycogen in the muscle under the influence of an anesthetic agent which has very little, if any, glycogenolytic action. Furthermore, the rate of injection was carefully controlled.

LITERATURE. The literature dealing with the effect of epinephrine on glycogen is too extensive to be reviewed here. Consequently only references pertinent to the subject will be mentioned.

Eadie (1929), working on cats under the influence of iso-amylethyl barbiturate (amytal), found that the glycogen in muscles was not significantly reduced one and a half hours after the subcutaneous injection of 1 mgm. of epinephrine. Firor and Eadie found specimens of muscle from cats under iso-amylethyl barbiturate taken an hour after the administration of epinephrine did not differ appreciably in glycogen content from those of the control series. On the contrary, Eadie (1929) found that after the subcutaneous injection of 0.1 mgm. of epinephrine to white rats a decrease of glycogen occurred, although this was not observed when larger doses were given. He was of the opinion that the difference between the rat and the cat, in regard to the reaction of epinephrine is a difference of species, and is not due to variations in dosage or in time of sampling. Soskin stated that the depletion of glycogen in muscles subsequent to injection of epinephrine, noted by many workers, may be accounted for by increased muscular activity. Houssay and Mazzocco found the glycogen content of suprarenalectomized rats to be lower than that of a control group.

In favor of the decrease of glycogen in muscles due to the injection of epinephrine, the following evidence may be cited: Blatherwick and Sahyun found that after the subcutaneous injection of 1 mgm. of epinephrine the

glycogen of muscles of rabbits was diminished. Agadschanianz found that glycogen disappeared completely from the muscles of fasting dogs which had received from 1.1 to 1.5 mgm. of epinephrine. Cori and Cori have demonstrated a constant decrease of glycogen subsequent to the administration of epinephrine. They also found that following such injections the lactic acid level of the blood was markedly elevated. Ohara found that the subcutaneous injection of 0.5 to 1 mgm. of epinephrine was followed by marked reduction in glycogen. Pollak noted that after repeated injections of epinephrine into rabbits glycosuria ceased and that the glycogen content of the skeletal muscles was reduced almost to zero. Ringer, Dubin and Frankel demonstrated that after the administration of epinephrine to phlorhizinized dogs the "residual glycogen" disappeared, and thus they were able to rid the muscles of glycogen. Sahyun and Luck stated their belief that following injection of epinephrine there is an initial period in which hyperglycemia develops and in which the liver and glycogen in muscles diminish, whereas in the latter phase of the action of the substance the glycogen in muscles continues to decrease, and the glycogen in liver increases. Ohara injected epinephrine intravenously into dogs with Eck fistulas, into head-thorax preparations and into dehepatized animals. In each group he obtained a rise in sugar in the blood and a decrease in glycogen in the muscles. Geiger and Schmidt found that injection of epinephrine into phlorhizinized animals causes decrease in glycogen in muscles and an increase of the lactic acid of the blood, the glycogen in muscles being transformed to lactic acid, and the latter in turn being converted into glycogen in the liver. Sachs demonstrated a slight but definite diminution of glycogen in muscles following administration of epinephrine.

METHODS AND MATERIALS. All experiments were performed on dogs under the influence of iso-amylethyl barbiturate. Previous work had demonstrated change in glycogen of muscles is only slight under this form of anesthesia (Major and Bollman).

Best, Hoet and Marks have noted that corresponding muscles of opposite extremities correspond closely in glycogen content. This observation has been repeatedly confirmed by Markowitz and Soskin, and we were able to confirm it also. Consequently, when experimental procedures were carried out on the glycogen content of a muscle, its fellow was used as a control, the control muscle being secured prior to subjecting the animal to the procedure in question. In a few cases a section of the same muscle was used as the control, the greatest care being taken to preserve the blood supply, the usual procedure being to use the distal end of the muscle as the control, the proximal end thus being insured a good supply of blood.

The epinephrine was injected intravenously in all the experiments, since it was felt that if injected subcutaneously the rate of absorption might not be uniform. The epinephrine was diluted to such an extent that 1.5

cc. entered the vein each five minutes of the experimental period in cases in which the drug was given slowly, the solution running into the vein by gravity from a graduated buret. In a few of the early experiments larger doses of epinephrine were injected quickly.

Glycogen was determined according to the method described by Pflüger, with slight modification.

RESULTS. *Effect of iso-amylethyl barbiturate on glycogen in muscles.* Although the influence of this anesthesia on glycogen in muscles was reported in a preceding paper it should be noted here that iso-amylethyl barbiturate has little, if any, effect on the glycogen content of skeletal muscle. A representative protocol follows.

From 8:35 a.m. to 8:50 a.m. 40 mgm. of iso-amylethyl barbiturate for each kilogram of body weight were injected intravenously into a dog weighing 22.5 kgm. The control specimens of muscle were removed from 9:09 a.m. to 9:15 a.m. At 10:30 a.m. eight additional milligrams of iso-amylethyl barbiturate for each kilogram of body weight were injected intravenously. The second set of specimens of muscle was removed from 11:30 a.m. to 11:34 a.m. Results were as follows:

MUSCLE	TIME	GLYCOGEN
		per cent
Right sartorius	9:09 a.m.	0.259
Left sartorius	11:30 a.m.	0.225
Right quadriceps	9:11 a.m.	0.376
Left quadriceps	11:31 a.m.	0.363
Right gracilis	9:14 a.m.	0.223
Left gracilis	11:32 a.m.	0.225
Right adductor	9:15 a.m.	0.329
Left adductor	11:34 a.m.	0.384

Effect of massive doses of epinephrine on glycogen in muscles. Five experiments were performed to determine the effect of large doses of epinephrine on glycogen in muscles. The doses varied from 1 to 2.5 mgm. each hour, over periods ranging from three to eighteen hours. In all such cases it was found that the glycogen content of the muscles was definitely diminished. Only one such experiment is given in detail, but it is typical of all those of this series.

From 8:20 a.m. until 8:50 a.m. 47 mgm. of iso-amylethyl barbiturate for each kilogram of body weight were injected intravenously into a dog weighing 19 kgm. After taking a set of specimens of muscle, which served as controls, epinephrine was injected intravenously continuously at the

rate of 2.5 mgm. each hour for a period of two hours. The results were as follows:

MUSCLE	TIME	GLYCOGEN
		per cent
Right sartorius	9:47 a.m.	0.439
Left sartorius	12:00 m.	0.270
Right adductor	9:46 a.m.	0.813
Left adductor	12:08 p.m.	0.728
Right gracilis	9:45 a.m.	0.515
Left gracilis	12:05 p.m.	0.299
Right quadriceps	9:44 a.m.	0.625
Left quadriceps	12:04 p.m.	0.420

Effect of rapid injection of 0.04 mgm. of epinephrine. To determine the effect of the rapid injection of 0.04 mgm. of epinephrine two experiments were performed. The results were similar, although different in the degree of glycogenolysis. In the first experiment, which is not given in detail, the glycogen was diminished 50 per cent in four hours. The second experiment is reported below. The dog used in this experiment weighed 23 kgm. and was under iso-amylethyl barbiturate anesthesia. After taking the first set of specimens of muscle the epinephrine was injected over a five-minute interval, from 11:15 a.m. until 11:20 a.m. Subsequently two other groups of specimens of muscle were taken. The results were as follows:

MUSCLE	TIME	GLYCOGEN
		per cent
Left sartorius	10:55 a.m.	1.020
Right sartorius	11:45 a.m.	0.576
Left sartorius	12:07 p.m.	0.689
Left quadriceps	10:56 a.m.	0.809
Right quadriceps	11:47 a.m.	0.664
Left quadriceps	12:08 p.m.	0.548
Left gracilis	10:57 a.m.	0.417
Right gracilis	11:49 a.m.	0.362
Left gracilis	12:10 p.m.	0.473
Left adductor	10:58 a.m.	1.350
Right adductor	11:51 a.m.	1.230
Left adductor	12:11 p.m.	0.693

Effect of rapid injection of 0.02 mgm. of epinephrine for each kilogram of body weight. Four experiments were performed using 0.02 mgm. of epinephrine for each kilogram of body weight. In all the decrease in glycogen in the muscles varied only slightly. The following is typical of the group.

The dog weighed 22.3 kgm. After anesthesia had been induced two control sets of specimens of muscles were removed. At 9:53 a.m. 0.02 mgm. of epinephrine for each kilogram of body weight was injected quickly by the intravenous route, with results as follows:

MUSCLE	TIME	GLYCOGEN
		per cent
Right sartorius	9:21 a.m.	0.817
Right sartorius	9:45 a.m.	0.838
Left sartorius	9:58 a.m.	0.793
Right sartorius	10:27 a.m.	0.669
Left sartorius	10:52 a.m.	0.707
Left sartorius	12:17 p.m.	0.647
Right adductor	9:23 a.m.	0.979
Right adductor	9:52 a.m.	0.957
Left adductor	10:14 a.m.	0.907
Right adductor	10:33 a.m.	0.829
Left adductor	10:56 a.m.	0.808
Left adductor	12:22 p.m.	0.839
Right quadriceps	9:24 a.m.	0.825
Right quadriceps	9:47 a.m.	0.846
Left quadriceps	10:12 a.m.	0.746
Right quadriceps	10:29 a.m.	0.754
Left quadriceps	10:52 a.m.	0.569
Left quadriceps	12:20 p.m.	0.501
Right gracilis	9:26 a.m.	0.632
Right gracilis	9:50 a.m.	0.619
Left gracilis	10:00 a.m.	0.611
Right gracilis	10:31 a.m.	0.572
Left gracilis	10:54 a.m.	0.569
Left gracilis	12:24 p.m.	0.385

When 0.008 mgm. of epinephrine for each kilogram of body weight was injected intravenously into a dog under iso-amylethyl barbiturate it was found that the average decrease in the glycogen in the four sets of muscles after an interval of one hour was 33 per cent.

Effect of continuous injection of 0.00016 mgm. of epinephrine for each kilogram of body weight for each minute. Three experiments were performed using 0.00016 mgm. of epinephrine for each kilogram of body weight each minute, and the results were found to be consistent. This was the smallest

dosage of the drug which was found to produce a distinct decrease in the glycogen content of skeletal muscle. A representative experiment follows:

The dog weighed 19 kgm. From 8:35 a.m. until 9:00 a.m. 47 mgm. of iso-amylethyl barbiturate for each kilogram of body weight were injected intravenously. During the period from 9:40 a.m. until 11:25 a.m. the animal received 0.00016 mgm. of epinephrine for each kilogram of body weight each minute. The results follow:

MUSCLE	TIME	GLYCOGEN
		per cent
Right sartorius	9:05 a.m.	0.526
Left sartorius	10:25 a.m.	0.392
Right sartorius	11:16 a.m.	0.375
Left sartorius	12:10 p.m.	0.318
Right quadriceps	9:07 a.m.	0.512
Left quadriceps	10:27 a.m.	0.384
Right quadriceps	11:17 a.m.	0.336
Left quadriceps	12:11 p.m.	0.297
Right gracilis	9:08 a.m.	0.309
Left gracilis	10:29 a.m.	0.256
Right gracilis	11:19 a.m.	0.234
Left gracilis	12:13 p.m.	0.234
Right adductor	9:10 a.m.	0.501
Left adductor	10:31 a.m.	0.334
Right adductor	11:20 a.m.	0.289

It is conceivable that the diminution of glycogen in muscles in the foregoing experiments was due to reduction in the blood supply of the muscles due to vasoconstriction. Therefore it seemed expedient to determine whether such a dose of epinephrine causes any change in arterial blood pressure or in volume of blood in a limb. Consequently studies were carried out on two dogs under iso-amylethyl barbiturate, in which arterial blood pressure tracings were done. In one of the animals simultaneous plethysmographic records were made, injecting 0.00016 mgm. of epinephrine for varying periods of time. In no case did this dose cause an elevation of arterial blood pressure or a diminution of limb volume. A final series of experiments was done on the effect of epinephrine on glycogen of muscles, using the dose of 0.000066 mgm. for each kilogram of body weight each minute. It was found that this dose had no effect on the glycogen content of the muscles of the animal injected.

COMMENT. It seems evident from the data presented that large doses of epinephrine have a definite glycogenolytic action, and that doses as small

as 0.00016 mgm. for each kilogram of body weight each minute also exert a glycogen depleting effect. The latter dose is considered to be within physiologic limits, as evidenced by the work of Cannon and Rapport and of Boothby.

The mechanism of the action of epinephrine remains obscure, but it would appear from the data presented that epinephrine does not promote glycogenolysis by effecting a reduction in the caliber of the vessels, particularly in those experiments in which minimal doses were used.

SUMMARY

Iso-amylethyl barbiturate was found to cause little, if any, alteration in the glycogen content of skeletal muscle.

Massive doses of epinephrine caused a marked decrease of glycogen in muscles. Doses of the substance as small as 0.00016 mgm. for each kilogram for each minute caused definite glycogenolysis, whereas 0.000066 mgm. for each kilogram of body weight each minute did not result in demonstrable alteration in glycogen. The former dose is not attended by elevation of the arterial blood pressure or diminution of limb volume.

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INHIBITION OF LACTIC ACID FORMATION IN BRAIN AND KIDNEY TISSUE PRODUCED BY INTRAVENOUS INJECTION OF SODIUM MONOIODOACETATE

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Received for publication May 16, 1932

In a previous paper (Haldi, 1932) an hypothetical explanation was offered for the characteristic different rates of lactic acid formation in the various tissues. It was suggested that these differences might be due to the presence in different concentrations in the tissues of one or more enzymes controlling lactic acid production. The present research puts this hypothesis to the test by carrying a step further the interesting studies of Lundsgaard (1930a) on the effect produced by sodium monoiodoacetate on lactic acid formation in the tissues. Lundsgaard observed that when monoiodoacetic acid neutralized with sodium carbonate was injected into frogs and guinea pigs there was no lactic acid formed either in contracting or excised muscle. He also found (1930b) that in moderate concentrations sodium monoiodoacetate inhibited the enzymatic hexose cleavage that occurs in alcoholic fermentation. On the other hand it had no effect on glycogenolysis, phosphagen cleavage nor on the enzymatic activities of invertase, ptyalin and catalase. Sodium monoiodoacetate therefore apparently has an inhibiting effect on specific enzymes. If the assumption is correct that the same enzymes control lactic acid production in the various tissues, then monoiodoacetic acid should inhibit lactic acid formation in brain and kidney as well as in muscle. Should it be proved that this is not the case it would be necessary to abandon, or at least materially modify the hypothesis attributing the characteristic rates of lactic acid formation in the various tissues to different concentrations of enzymes.

METHODS AND RESULTS. Small dogs of 3 to 6 kilos were used in all the experiments. The animal was decapitated with a T-shaped guillotine, the brain and kidney excised and lactic acid analyses made in the manner described elsewhere (Haldi, 1932).

Since the animals used for studying the effects of sodium monoiodoacetate were anesthetized with morphine and urethane, nine experiments were performed as controls to establish the initial lactic acid content of the brain and kidney of anesthetized dogs and also the accumulation of lactic acid after ten minutes' incubation of the organs. Each animal was injected subcu-

taneously with one gram urethane and 0.4 cc. of a 2 per cent morphine solution per kilo body weight two to four hours before it was decapitated. The initial lactic acid content of the brain in eight out of nine control experiments varied between 50.1 and 60.4 mgm. per 100 grams of tissue. In one experiment it was slightly lower at 45.8 mgm. The average for the nine experiments gave an initial lactic acid content in brain tissue of 54 mgm. per cent 13 seconds after decapitation. In the kidney the initial lactic acid content was 14.5 to 35.1 mgm. per cent with an average of 22.9 mgm. per cent. The average time interval between decapitation and immersion of the kidney in liquid air was 17 seconds. The amount of increase in lactic acid was determined by taking the difference between the percentage content of the second portion of the brain incubated for ten minutes and

TABLE 1

Lactic acid content in milligrams per cent of brain and kidney after intravenous injection of sodium monoiodoacetate

EXPERIMENT	LACTIC ACID (MGM. PER CENT) OF BRAIN DROPPED IN LIQUID AIR 7 TO 42 SECONDS AFTER DECAPITATION	LACTIC ACID (MGM. PER CENT) OF BRAIN INCUBATED 10 MINUTES	LACTIC ACID (MGM. PER CENT) OF KIDNEY DROPPED IN LIQUID AIR 12 TO 59 SECONDS AFTER DECAPITATION	LACTIC ACID (MGM. PER CENT) OF KIDNEY INCUBATED 10 MINUTES	MILLIGRAMS MONOIODOACETIC ACID PER KILO BODY WEIGHT
10	62.0	146.9	15.6	15.6	65.8
11	45.8	141.0	19.8	16.0	68.9
12	43.2	107.8	22.7	15.9	42.8
13	41.3	94.7	20.5	20.6	82.1
14*	41.1	52.0	29.2	25.4	78.5
15	36.2	42.8	26.5	24.5	126.0
16*	31.5	33.9	12.4	10.6	72.2
17*	41.2	41.1	41.4	36.6	49.0

* Animal's heart had stopped beating approximately 1 to 2 minutes before decapitation.

that of the first portion frozen in liquid air immediately after decapitation of the animal. The increase of lactic acid in the anesthetized brain in ten minutes ranged from 89.9 to 142.8 mgm. per cent with an average of 113.3 mgm. per cent. The absolute amount of lactic acid in the brain at the end of approximately ten minutes varied between 140.9 and 192.9 mgm. per cent. In the kidney the increase of lactic acid at the end of ten minutes determined the same way as for brain tissue ranged from 25.2 to 53.7 mgm. per cent with an average of 36.3 mgm. per cent. The absolute amount of lactic acid in the kidney of anesthetized dogs at the end of approximately ten minutes' incubation of the organ was 45.8 to 80.6 mgm. per cent, the average for nine experiments 59.3 mgm. per cent. From these control experiments it was therefore clearly established that excised brain and

kidney from dogs anesthetized with morphine and urethane show a marked increase in lactic acid after 10 minutes' incubation of the tissues.

The results obtained after injection of sodium monoiodoacetate are given in the table. Monoiodoacetic acid was neutralized with sodium carbonate and the solution injected slowly into the femoral vein. It was found in conformity with Lundsgaard's observations (1930) that sodium monoiodoacetate is highly toxic. Susceptibility to toxicity however varies markedly in different animals. In experiment 14 the injection was begun at 10:30 a.m. and continued slowly for approximately twenty-five minutes. At 10:58 the animal was dead, having received 78.5 mgm. monoiodoacetic acid per kilo body weight. In striking contrast, the animal in experiment 15 received 126 mgm. per kilo body weight and was still alive when brought to the guillotine 45 minutes from the time the injection was begun. In this experiment as in the previous one the solution was injected slowly. In experiments 10, 11, 12, 13 and 15 the animal was alive although in poor condition immediately prior to decapitation. The animal was dead in experiments 14, 16, and 17. In these latter experiments one to two minutes elapsed between cessation of the heart beat and removal of the brain and kidney.

A marked difference is observed in the effects produced in the brain and kidney. In no instance was there any formation of lactic acid in the kidney at the end of ten minutes' incubation. On the other hand, in every experiment except 10 and 13 the lactic acid content of the kidney decreased after incubation. In the case of the brain however experiments 10, 11, 12, and 13 show a fairly large increase in lactic acid whereas in experiments 14, 15 and 16 the increase was insignificant and in experiment 17 there was no increase. In experiments 10 and 11 the absolute amount of lactic acid in the brain and the amount formed in ten minutes fall within the limits of the values obtained in the control experiments. In experiments 12 and 13 lactic acid increased during incubation but the amount formed was much less than in the control experiments. Although the concentration of sodium monoiodoacetate was not sufficient to prevent completely the formation of lactic acid, it nevertheless had a marked inhibitory effect. In experiments 14, 15 and 16, sodium monoiodoacetate produced almost complete inhibition of lactic acid. In these experiments lactic acid which normally increased 80 to 100 mgm. per cent in ten minutes increased only 2.4 to 10.9 mgm. per cent. In contrast with the results of the experiments on the kidney the final lactic acid values of the brain were in no instance lower than the initial values. It is interesting to note that there was a complete inhibition of lactic acid formation in the excised brain in only one experiment. In this experiment the dosage was sufficient to kill the animal.

Discussion. Sodium monoiodoacetate in sufficient concentration in-

hibited lactic acid formation in both brain and kidney tissues. Assuming a uniform distribution in the tissues of the injected sodium monoiodoacetate, an amount large enough to inhibit lactic acid formation in the kidney did not always suffice to produce the same effect in the brain. This is what might be expected in accordance with the hypothesis attributing differences in the rate of lactic acid formation in the various tissues to differences in the concentration of one or more enzymes controlling lactic acid formation. A greater amount of sodium monoiodoacetate should be required to counteract the greater amount of enzyme in the brain. These observations, therefore, while they do not offer final proof, nevertheless lend support to the supposition of an enzymatic control of lactic acid formation in the various tissues. In this connection it is of interest to note that Dudley (1931) has reported inactivation of glyoxalase by sodium monoiodoacetate.

The average loss in eight experiments of 2.9 mgm. per cent lactic acid in the kidney raises an interesting question. Can it be accounted for as an oxidation by the oxygen contained in the blood trapped in the excised organ? A similar loss of lactic acid in excised muscle was observed by Lundsgaard (1930a). Theoretically, one milligram of lactic acid should be oxidized by 746 c.mm. of oxygen or 1.34 mgm. by 1 cc. of oxygen. Meyerhof and Himwich (cited by Himwich, Koskoff and Nahum, 1930) found experimentally that 1 cc. of oxygen caused the disappearance of 1.6 to 2.7 mgm. lactic acid in the excised muscle of the rat. Apparently some of the lactic acid was converted into the precursor state. Applying these experimental deductions on the removal of lactic acid in muscle to kidney tissue a maximum of 1.8 cc. or a minimum of 1.07 cc. oxygen would have been required for the removal of 2.9 mgm. lactic acid per 100 grams of kidney tissue. On this basis, assuming that the blood in the excised tissues contained 16 to 18 volumes per cent oxygen, the oxidation would have been effected by a maximum of approximately 10.6 or a minimum of 6.3 volumes per cent of blood. It is possible that the excised kidney contained sufficient blood, in accordance with the above calculations, to account for the observed loss of lactic acid. The actual amount of blood in the tissue, however, was not ascertained.

SUMMARY

Intravenous injections of monoiodoacetic acid neutralized with sodium carbonate inhibited lactic acid formation in excised brain and kidney of dogs anesthetized with morphine and urethane.

In a series of control experiments with no injection of monoiodoacetic acid lactic acid in the brain of dogs showed an increase of 89.9 to 142.8 mgm. per cent with an average of 113.3 mgm. per cent after ten minutes'

incubation. The increase in lactic acid after incubation of the kidney was 25.2 to 53.7 mgm. per cent with an average of 36.3 mgm. per cent.

The injection dosage of 42.8 to 126 mgm. monoiodoacetic acid per kilo body weight sufficed in every instance to prevent formation of lactic acid in the kidney. With the brain the results varied from a complete inhibition to no inhibition at all. In several experiments lactic acid was formed but there was a marked reduction in comparison with the controls.

These observations are interpreted as evidence in support of the hypothesis attributing differences in the rate of lactic acid formation in the various tissues to different concentrations of enzymes controlling lactic acid production.

A loss of lactic acid was observed in the kidney after incubation. It is suggested that lactic acid might have been oxidized by the oxygen contained in the blood that was trapped in the excised organ.

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A METHOD FOR MEASURING ELASTICITY IN VIVO AND RESULTS OBTAINED ON THE EYEBALL AT DIFFERENT INTRAOCULAR PRESSURES

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Received for publication May 17, 1932

Theory of elasticity measurements in vivo. The general expression for any coefficient of elasticity is the quotient obtained by dividing the stress by the strain. In the case of the coefficient of volume elasticity the stress is the change in pressure (dP) and the strain is the change in volume dV divided by the original volume V, or E = coefficient of volume elasticity = $dP \Big/ \frac{dV}{V} = V \Big/ \frac{dV}{dP}$. This is the formula for the bulk modulus and strictly speaking should not be applied to the determination of the coefficient of elasticity where there is no compression or dilatation of the contents under pressure change. But it is found that, in physiological systems, wherever the elasticity of the membranes and vascular bed permits a volume change (dV) as a result of pressure change (dP), the formula $E = V \Big/ \frac{dV}{dP}$ gives a value for E which may be taken as a measure of the general elasticity of the system as a whole. This coefficient E is neither the bulk modulus nor Young's modulus (coefficient of linear stretch) but is a measure of the general functional elasticity of the system.

This method has been successfully applied to determine the elasticity of the dural sac and its contents (Weed, Flexner and Clark, 1932a, b). In these experiments cerebro-spinal fluid pressure was measured on dogs under ether anaesthesia by puncture into the sub-arachnoid space through the occipito-atlantoid ligament. The needle was connected to a bubble manometer and to open-end manometers of different bores, and pressure readings were taken first with the animal in the horizontal position and then when tilted to a vertical, head-down or tail-down, position. When the animal is tilted there is a rise in pressure in the head-down position and a fall in pressure in the tail-down position and the elasticity of the membranes and vascular bed permits an internal shift of fluid from one part of the nervous system to another as well as an external dislocation into or from the open-end manometers. As larger manometers are used there is greater external dislocation of fluid and consequently less pressure change on tilting.

The relation of these external volume and pressure changes to the elasticity of the system can be explained by means of the simplified diagram of the dural sac and its contents given in figure 1. When the animal is tilted, head-down, there is an internal shift of fluid due to collapse of the upper and stretch of the lower parts of the elastic membranes. Owing to the rigid character of the bony sheath and reciprocal readjustment in the vascular bed this shift is limited in extent. The upper part of the system is however capable of collapsing further after the lower part has stretched as far as possible and P_0, the increase in pressure measured with the bubble manometer when the animal is tilted, is the pressure necessary to prevent further collapse with external dislocation of fluid. When a 1 mm. bore manometer is used the upper part of the system collapses to the extent of dislocating a volume dV_1 into the manometer and P_1, the pressure change recorded with this manometer, is the pressure necessary to prevent dislocation of more fluid.

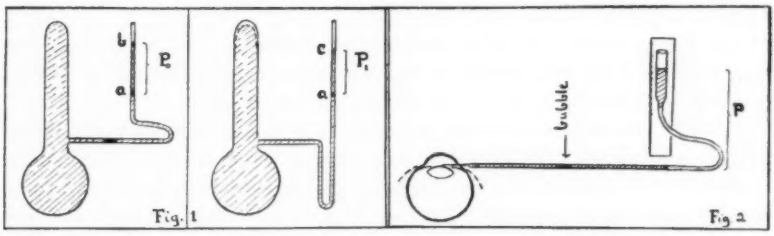

Fig. 1. Diagram of dural sac and contents. a = pressure reading of cerebrospinal fluid in horizontal position. b = pressure reading on tilting to vertical head-down (bubble manometer). c = pressure reading on tilting to vertical (open-end manometer).

Fig. 2. Method of measuring intra-ocular pressure with bubble manometer.

As manometers of larger bore are used more fluid is dislocated and less pressure is needed to prevent dislocation of the remainder so that smaller and smaller pressure changes are recorded on tilting until the limit of collapse of the upper membrane is reached.

$P_0 - P_1 = dP_1$ = difference in pressure necessary to prevent dislocation of fluid dV_1 = amount dislocated into manometer (1).

$P_0 - P_2 = dP_2$ = difference in pressure necessary to prevent dislocation of fluid dislocated into manometer (2) = dV_2.

If the bubble manometer is not used $P_2 - P_1$ = the pressure change sufficient to prevent dislocation of fluid amounting to the difference between that dislocated into manometer (2) and that dislocated into manometer (1).

When a series of manometers was used it was found that dV_1/dP_1 =

$dV_2/dP_2 = dV_3/dP_3$, etc., giving a constant value for dV/dP in any one animal. By means of reciprocal compensation in the vascular bed the internal readjustment in volume on tilting was apparently kept constant enough throughout the range of the experiments to insure a constant relationship between external volume and pressure changes.

Since there are many elastic elements in the system (membranes and vascular bed) the ratio dV/dP cannot be directly related to the coefficient of linear stretch of any of them but it was found that when it is substituted in the formula $E = V\Big/\frac{dV}{dP}$, where V is the intradural volume, a value is obtained for E which is very constant for animals of the same age and species and which may be taken as a general coefficient of elasticity of the system as a whole. For instance in two dogs of the same age but different size it was found that $E = V\Big/\frac{dV}{dP} = \frac{63.1}{0.154} = 409$ in one dog and $E = \frac{94.4}{0.230} = 410$ in the other. In order to give the value of E in C. G. S. units the pressure, which is read in centimeters of saline, must be expressed as dgh = density × acceleration of gravity × height (cm.), or $E = 409 \times 1.006 \times 980 = 4.03 \times 10^5$ dynes per square centimeter.

Results obtained in this way are given in detail in other papers (Weed, Flexner and Clark, 1932a, b). In a large series of dogs E (the general coefficient of elasticity of the dural sac and its contents) was found to be constant in animals of approximately the same age which indicates a very constant elastic response to posture change in the cerebro-spinal fluid system over a fairly wide pressure range. When young and old dogs were compared the value of E was found to range from 4.56×10^5 dynes per square centimeter in puppies to 3.81×10^5 dynes per square centimeter in old animals. As E is a measure of resistance to deformation the decrease in E with age indicates greater extensibility in the older group.

The elasticity of the eyeball under different intraocular pressures. This method for determining the coefficient of elasticity can be applied to other parts of the body and is particularly well adapted to a study of the elasticity of the eyeball.[1] By assuming the eye to be a sphere, and the enclosing membranes the only elastic elements, the change in volume with pressure can be used to calculate both the general coefficient E and the coefficient of linear stretch (Young's modulus) of the membranes which can be called M. In a sphere with elastic walls filled with fluid $M = \frac{3}{2}\frac{r}{t} V\Big/\frac{dV}{dP}$,

[1] Through the courtesy of the Department of Anatomy animals which had been used in experiments on cerebro-spinal fluid pressure were subsequently used for measurement of elasticity of the eyeball and formed the majority of the animals used in these experiments.

where r is the radius and t the thickness of the membranes. If the ratio r/t is constant in eyes of different size, as was found to be the case, E is directly proportional to M.

METHOD. A 23 gauge needle was attached to a calibrated glass capillary tube by means of a ground glass joint. The capillary was connected to a movable reservoir by rubber tubing and, with the system filled with Locke solution, a bubble was introduced into the capillary at the tubing joint. By means of a clamp on the tubing the bubble was allowed to move slowly down the tube and the needle was introduced into the anterior chamber at the edge of the sclera with fluid flowing slowly from it and a counter pressure approximately equal to the intra-ocular pressure (see fig. 2). The bubble moved freely in the capillary tube with change in height of the reservoir and several series of readings were made of the position of the bubble at different pressures ranging from 100 to 450 mm. of saline. The change in volume corresponding to a given pressure change was calculated from the change in position of the bubble and the bore of the capillary. The ratio of the change in volume in cubic centimeters to the change in pressure (cm. saline) gave the values of dV/dP given in the tables. Whenever possible observations were made in the same eye while the animal was living and immediately after death. At the end of the experiment the eye was excised and the volume V of the contents was measured. The formula for M given above assumes a very thin membrane. In the eye the coats have an appreciable thickness and it is uncertain whether V should be the volume of the contents or the total volume of the eye. In the results given here V is taken to be the volume of the contents of the sphere, r the internal radius and t the average thickness of the enclosing membranes. If V is taken to be the total volume of the eye the values of E and M are increased approximately 50 per cent. The volumes of the whole eye and the contents were determined by the cubic centimeters of water displaced by the whole eye and by the coats. The average external and internal radii were calculated from the volumes and the difference gave the average thickness.

RESULTS. Under ideal conditions there was no appreciable difference in the values of dV/dP obtained with the living eye and the eye (in situ) immediately after death (see table 1). The secretion and absorption either balanced each other or took place too slowly to affect the results. This was always true in the macaques, and usually in the dogs. In cats on the other hand there was frequently an active and rapid secretion of aqueous when the needle was inserted, possibly in response to injury. When this occurred dV/dP could not be obtained in the living eye but in five cats comparable results were obtained with the animal living and dead for one eye at least.

The value for $\frac{dV}{dP}$ would differ from animal to animal owing to differences

in eye volume even if the elasticity were the same. There are undoubtedly variations in elasticity from eye to eye in the same species but the variations from the average were not very great except at high pressures where the small change in volume as well as the possibility of filtration and absorption make the results less accurate.

The results given in table 1 show that dV/dP is not constant throughout the pressure range used and consequently the coefficient of elasticity varies with the pressure applied. The large change in volume with pressure at low pressure ranges indicates collapse of the coats and the small value of dV/dP at high pressures indicates a greater E or greater resistance to deformation. Consequently one cannot give one value for E in the case of the eyeball but must plot the results in a curve showing the average E for each pressure range. ($E = V/\frac{dV}{dP} \times 980 \times 1.006$ = general coefficient of elasticity in dynes per square centimeter.) The results were

TABLE 1

Values of dV/dP at different intraocular pressures

PRESSURES, MM. SALINE	MACAQUE (C 30) dV/dP		DOG (C 38) dV/dP		CAT (C 50) dV/dP	
	Living	Dead	Living	Dead	Living	Dead
100–150	0.0095	0.0100		0.019	0.0157	0.0137
150–200	0.0067	0.0062	0.019	0.0164	0.0158	0.0125
200–250	0.0065	0.0062	0.0138	0.0128	0.0129	0.0115
250–300	0.0034	0.0030	0.0108	0.0086	0.0092	0.0096
300–400	0.0016		0.0054	0.005	0.0062	0.0087
400–500			0.0050		0.0043	0.0044

averaged for living and dead animals and the values given in figure 3 represent a series of 20 dogs, 8 macaques (4 *Pithecus sinicus*, 4 *Pithecus rhesus*) and 12 cats.

The coefficient of linear stretch $M = (3r/2t)\ E$. The average value of $3r/2t$ was found to be 9.7 in cats, 10.7 in dogs and 14.2 in the macaque. The values for M are given in figure 4 and show approximately equal coefficients for dogs and cats and a higher value in the macaque, so that throughout the pressure range used the eye of the macaque is less extensible than the eyes of dogs and cats.

Young's modulus was also determined directly on strips of cornea and sclera (5 × 15 mm.). The results were less satisfactory than those obtained by means of volume and pressure changes but were of the same order of magnitude and showed that the cornea and sclera have approximately the same degree of elasticity.

Values for change in volume with pressure have been obtained by other observers (Koster, 1901; Ridley, 1930) but have not been used to calculate

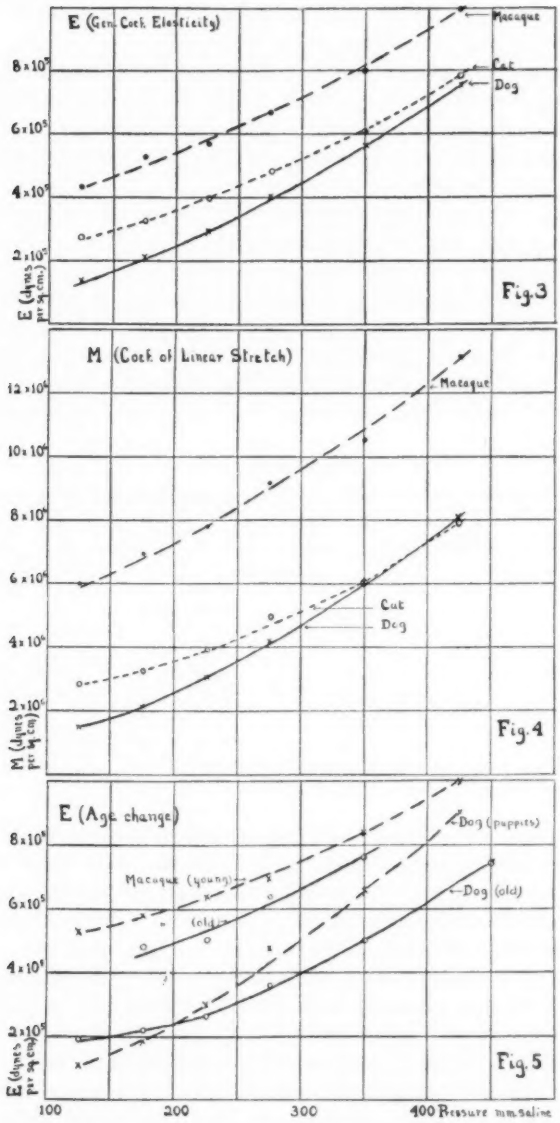

Fig. 3. E (general coefficient of elasticity of the eye as a whole).
Fig. 4. *M* (coefficient of linear stretch of the coats of the eye).
Fig. 5. *E* (general coefficient of elasticity) compared in young and old animals.

the coefficient of elasticity. When E is calculated from their results it falls in line well with the values given in figure 3.

Results were also gotten with human eyes in two cadavers (dead two weeks). They gave very high values for E, doubtless much higher than the living eye, for in one dog E was determined immediately after death and two weeks later and the value was found to have doubled. These results are given in table 2.

The results for the elasticity of the dural sac and its contents showed a decrease in E with age (Weed, Flexner and Clark 1932b). The elasticity of the eye showed a less consistent variation with age but the values of E for 5 old dogs and 9 puppies are compared in figure 5 as well as the results for 4 adult and 4 juvenile macaques. Apparently in old dogs, as compared with puppies, the eye is less collapsible at low pressures but more extensible (lower E) at high pressures. Such a difference might be due to a change in the ratio r/t rather than to a change in M, the coefficient of linear stretch

TABLE 2

General coefficient of elasticity E in human eye and dog's eye (dead two weeks)

PRESSURE, MM. SALINE	E IN DYNES PER CM.^{2}		
	Dog C 45		2 human eyes dead 2 weeks
	Living	Dead 2 weeks	
100–150		3.75×10^5	13.1×10^5
150–200	1.90×10^5	5.54×10^5	18.7×10^5
200–250	2.40×10^5	7.33×10^5	19.8×10^5
250–300	4.23×10^5	8.55×10^5	22.8×10^5
300–400	6.51×10^5	10.88×10^5	26.2×10^5
400–500	8.59×10^5	11.17×10^5	34.2×10^5

of the eye coats. But r/t did not vary in dogs so that there is a change in M with age proportional to the change in E shown in figure 5. In the macaque there is a greater extensibility (lower E) in the adult eye throughout the pressure range investigated. The ratio r/t did not vary appreciably so that this indicated a lower value of M in the adult eye.

The change in elasticity with pressure is interesting in relation to clinical problems. The eye normally undergoes large variation in pressure with muscular activity and blood pressure changes. In glaucoma, when the eye is under high pressure, it is less able to compensate for pressure changes by volume changes owing to the increase in E (resistance to deformation) at high pressures. When glaucoma is found associated with relatively normal intraocular pressure the eye may have a coefficient of elasticity greater than normal and it is possible that a high coefficient of elasticity may be a factor in the etiology of glaucoma.

SUMMARY

A general method is described for determining the coefficient of elasticity in vivo by simultaneous measurements of volume and pressure changes. This method was applied to a study of the elasticity of the eyeball at different pressures. A needle, connected with a bubble manometer, was inserted into the anterior chamber of living and dead eyes in situ. Change in position of the bubble gave the volume changes as the pressure varied.

It was found that, if no abnormal secretion of aqueous took place in response to injury, the values of the coefficient of elasticity were the same in living and dead eyes (immediately after death), and varied throughout the pressure range used (100–450 mm. saline). The eye has a greater resistance to deformation (larger E) at high pressures. Values of E, the general coefficient of elasticity of the eye as a whole, and M, the coefficient of linear stretch of the eye coats, are given for the dog, cat, and macaque, for both young and old animals.

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THE PRODUCTION OF FUNCTIONAL CORPORA LUTEA BY THE DIRECT INTRAFOLLICULAR INJECTION OF EXTRACTS OF PREGNANCY URINE[1]

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Received for publication May 18, 1932

In a recent publication it was indicated that the direct intrafollicular injection of small quantities of extracts of urine of pregnancy resulted in the luteinisation of the injected follicles (Friedman, 1932a). By the use of such technique it was possible to produce corpora lutea in one ovary of an oestral rabbit without causing any discernible change in the ripe follicles of the contralateral, untreated ovary. There were two considerations, however, which materially detracted from the theoretical significance of these findings. In the first place, the corpora lutea produced by the intrafollicular injection did not manifest any functional activity. They neither prevented the onset of heat or ovulation, nor sensitized the uterus for the production of decidual tumours, despite the fact that they grossly resembled normal corpora lutea. In addition to this consideration of the apparent functional deficiencies of the unilateral corpora lutea, was the question of the value of the unruptured follicles in the untreated ovary for the detection of changes in the concentration of gonad-stimulating material in the blood stream. It was conceivable, for example, that ruptured follicles might be able to utilise gonadotropic material more efficiently than unruptured follicles, so that low concentrations of such material might be able to effect the luteinisation of ruptured follicles without having any demonstrable effect upon the ripe, unruptured follicles of the contralateral ovary.

The present experiments may be divided into two series. The first series was designed to determine which of the several possible factors was chiefly responsible for the functional deficiencies of the unilateral corpora lutea, so that the factor, or factors, in question could be circumvented, and so that finally functional unilateral corpora lutea could be produced. The second series of experiments was performed in an attempt to evaluate the

[1] This work was aided by a grant from the Committee on Research in Problems of Sex of the National Research Council. It is also a pleasant duty to acknowledge the generous coöperation of Doctors Kamm and Bugbee of the Parke Davis Laboratories.

worth of the unruptured ripe follicles as criteria of changes in the concentration of gonad-stimulating substances in the blood stream.

METHODS AND MATERIALS. As in our previous experiments post partum female rabbits were used exclusively. The extracts employed were prepared from pregnancy urine by Parke, Davis & Co., and were supplied in sterile bottles of 10 cc. capacity. These extracts were repeatedly subjected to bioassay by the rabbit method (Friedman, 1932b), so that the strength of each extract was known at the time of each intrafollicular injection. The material was kept in the ice chamber at all times except when in use. The technique of the intrafollicular injections was the same as that employed in the earlier experiments (Friedman, 1932a). In the present experiments, however, the volume of the extract discharged from the syringe was measured at the time of operation.

RESULTS. *Series I. Group A. An examination of the influence of the contralateral, untreated ovary.* In view of the established antagonism between the secretions of ripe follicles and corpora lutea (Leonard, Hisaw, and Fevold, 1932; Hisaw and Leonard, 1930; Courier, 1930a, b; Reynolds, 1932) there existed the possibility that secretions from the ripe follicles of the contralateral, untreated ovary interfered peripherally with the secretions of the unilateral corpora lutea. Moreover, it seemed possible that the structures in the untreated ovary jeopardised the optimal growth of the unilateral corpora lutea by competing with the latter for some essential element in the blood stream. Because of these considerations it was decided to remove the untreated ovary 24 hours after the intrafollicular injection.

Accordingly, all of the ripe follicles in one ovary of each of twenty rabbits were injected with a small quantity of an extract of urine of pregnancy. On the following day the untreated ovary was extirpated and examined for the presence of freshly ruptured follicles. Each of these rabbits was then subjected to a third operation two to five days later (on the third to sixth day after the intrafollicular injection), at which time several linen threads were drawn through the uterus. At autopsy six days afterwards the remaining ovary and uterus were carefully examined grossly, and then immediately placed in formalin for subsequent histological examination.

A period of nine weeks elapsed between the first and last of these twenty experiments. Repeated bioassays of the extract employed showed a gradual deterioration from 65 rat units per cubic centimeter to 50 rat units per cubic centimeter during the course of these experiments. The amount of the extract discharged from the syringe in the execution of the intrafollicular injections in any one animal was usually between 0.02 and 0.03 cc. and in only one case did the amount exceed 0.04 cc. In not one of the twenty rabbits did the intrafollicular injection result in ovulation from the

untreated ovary. (Minimal intravenous ovulating dose of this extract was from 0.05 to 0.09 cc. per rabbit, depending upon the weight of the animal.)

In nineteen of these twenty experiments the intrafollicular injection was successful, the injected ovary presenting from one to four corpora lutea when examined at autopsy. In seventeen of these successful experiments the nodular swellings found at the site of the thread in the uterus proved to be merely nodules of edematous uterine mucosa without a trace of decidual tissue. Yet, microscopic examination of the tumours in the remaining two of the nineteen rabbits revealed genuine decidual tissue. Grossly, the corpora lutea in these two rabbits were as large and as vascular as the ordinary corpora lutea of pseudopregnancy, and microscopically too, they were indistinguishable from the latter.

It is true that the corpora lutea in these two animals were among the best in this series, and that on the whole the corpora lutea produced in the present experiments were distinctly better than those produced in the earlier work (Friedman, 1932a) where the untreated ovary was not removed. Nevertheless, if one would examine the gross and microscopic appearance of the corpora lutea produced in the nineteen animals of the present series, he would find no good correlation between the character of the corpora lutea and the reaction of the uterus to the thread. In nine animals in which no decidual tumors were formed, the corpora lutea were at least as large, and as vascular, and as sound microscopically as the lutein bodies in the two animals in which deciduomata appeared. Nor was there any correlation between the number of good corpora lutea produced and the formation of a decidual tumor. In the two instances in which the corpora lutea proved to be functional there were three corpora lutea in one animal, and four in the other. In the nine instances wherein the corpora lutea appeared to be quite as good as the functional corpora lutea, there were four animals with two corpora lutea; four, with three good corpora lutea; and one animal in which four good corpora lutea were produced.

Finally, there appeared to be no correlation between the strength of the extract at the time of the irrigation and the functional activity of the resulting corpora lutea, insofar as the two experiments in which functional corpora lutea were produced were in the middle of the series. Nor did the quantity of extract used for the injection appear to make any difference; the quantity used for the production of the functional unilateral corpora lutea being between 0.02 and 0.03 cc. in each case.

B. The regularity of the decidual reaction in the presence of normal corpora lutea. It was assumed at the start of these experiments that decidual tumors could be produced regularly in the presence of functional lutein tissue. The apparent lack of correlation between the structure and the functional activity of the corpora lutea produced in the preceding experiments raised some doubt as to the adequacy of my particular technique for

the production of deciduomata, and made it imperative to put this technique to a satisfactory trial.

Consequently, thirteen female rabbits were given a single intravenous injection of an active extract. Ovulation was verified in each rabbit by inspection at laparotomy on the following day. It so happened that in two of these thirteen rabbits all the corpora lutea save one were in one ovary. In each of these two rabbits the ovary containing most of the corpora lutea was removed, leaving behind only one corpus luteum. Four days later the uterus in each one of these rabbits was slit open along the free border and three linen sutures passed through the muscular layer and mucosa, and tied loosely in place. The technique was exactly that executed in the 20 rabbits of the preceding experiments (A). At autopsy, six days afterwards, nodular enlargements were found at the site of the thread in each uterus. Microscopically the nodules proved to be genuine deciduomata in eleven of the thirteen rabbits. In the remaining two rabbits the nodules consisted entirely of edematous mucosa. It was in these two rabbits, however, that only one corpus luteum was left in place. From gross inspection, the single corpus luteum in each of these two animals was as large and vascular as the corpora lutea in eleven other animals of this group, and was quite comparable to the corpora lutea found at a similar stage of pseudopregnancy resulting from a sterile coitus.

In view of Corner's results with ovarian extirpation during early pregnancy (1928) one might have expected that one corpus luteum would be sufficient to sensitize the uterus for the production of decidual tumors. It is, of course, possible that it was mere coincidence that the only two failures in this series of experiments occurred in the animals in which there was only one corpus luteum. It is more probable, however, that more of the lutein hormone is required for uterine sensitization than is required for the proliferative change. At any rate, it appears evident that the many unsuccessful attempts to provoke the decidual reaction in the experiments of group A cannot be charged to the threading technique or to an irregularity of the decidual reaction.

C. The influence of the operative procedures on corpora lutea produced by intrafollicular injection. After the results in the first two groups of experiments were examined, the factor of operative trauma was more seriously considered as an important element in causing the failure of functional manifestation on the part of most of the corpora lutea produced by intrafollicular injection In order to evaluate this factor, the follicles in one ovary of seven rabbits were injected with small quantities of an active extract just as in A. At the end of the operation each rabbit was given intravenously one minimal effective dose of extract (about one rat unit per kilo, or 0.02 cc. per kilo of the extract in question). This dosage will provoke ovulation from ripe follicles, but will produce neither ovulation

nor luteinisation in unripe follicles (Friedman, 1932b). On the following day the contralateral, untreated ovary was removed, and it was noted that in each case ovulation had occurred. Five days later the uterus was threaded in all the females of this group, and six days after this operation the animals were sacrificed. In five of these females the injected ovary contained several corpora lutea. In the remaining two does the injected ovary contained neither corpora lutea, corpora hemorrhagica, nor large follicles. Of the five rabbits in which corpora lutea were found decidual tumors were found in only two of them, despite the fact that the corpora lutea in the three other rabbits appeared, grossly and microscopically, to be equally as good. It must be admitted that there were more corpora lutea in the ovaries associated with decidual tumors (six in one ovary, seven in the other) than in the ovaries associated with no decidual reaction (three, three and four). Nevertheless, when one considers that the intravenous injection of a minimal effective dose of an extract of urine of pregnancy results not only in ovulation, but in the development of functional corpora lutea, it is clear that the data obtained in the above experiments give strong support to either or both of two possibilities; namely, 1, that the operative procedures involved in direct intrafollicular injection prevent the growth and development of lutein tissue in an entirely normal manner, even after the application of a stimulus known to be adequate for the normal development of functional lutein tissue (intravenous injection of an active urine extract), or 2, that these procedures somehow prevent the manifestation of full functional activity of normal lutein tissue.

D. The production of functional corpora lutea unilaterally without the extirpation of the untreated ovary. The revelation that a degree of trauma, insufficient to be detected by gross or microscopic examination, could prevent the full functional activity of corpora lutea made it necessary to reexamine the influence of the untreated ovary. It was still possible that the presence of the untreated ovary was the most important factor concerned, and that the factor of trauma appeared as a significant one only when the untreated ovary, with its large follicles, had been removed. But it was now quite as likely that all the results with intrafollicular injections so far could be adequately explained by unappreciated variations in the degree of operative trauma.

To test this hypothesis twelve rabbits were subjected to unilateral intrafollicular injections of an active extract just as in A, with the exception that the untreated ovary was not removed, but allowed to remain in the animal for the duration of the experiment. In each case the uterus was threaded seven days after the first operation, and autopsy performed six days after that. In these experiments the extract employed assayed 65 rat units per cubic centimeter, and the amount of material discharged from the syringe at any one operation did not exceed 0.03 cc.

In eight of these rabbits from one to four corpora lutea were found in the injected ovary at autopsy; in the remaining four animals the intrafollicular injection proved to be completely unsuccessful. In five of the eight successful experiments the corpora lutea found in the injected ovary were distinctly smaller and less vascular than the corpora lutea produced in most of the experiments in group A where the untreated ovary was removed. In the remaining three instances the corpora lutea were as good as the best produced in group A. In two of these three instances decidual tumors were found at the side of the threads. The number of corpora lutea found in the injected ovary of each of these two animals was four. Decidual tumors were found in the uterus of still another animal, however. In this case also the number of corpora lutea was four, but they were distinctly paler and smaller than the corpora lutea seen in the three animals referred to above.

In not one of the twelve rabbits did the follicles of the untreated ovary show any change, grossly or microscopically. The largest follicles in these ovaries did not exceed the size of the largest follicles in an oestral rabbit (1.4 mm.). Microscopic examination showed the granulosa to be intact, and without a trace of lutein transformation. The theca interna was not thickened. In no case was a blood follicle found.

Briefly, the corpora lutea produced in these experiments were not as uniformly good as the corpora lutea produced in group A. Nevertheless the incidence of functional corpora lutea in this group (3 out of 8 cases) was not lower than in A where the untreated ovary was removed (2 out of 19 cases). It is therefore apparent that the presence or absence of the untreated ovary is not of great significance in the production of functional corpora lutea by intrafollicular injection.

Series II. A. The effect of unilateral intrafollicular injections of saline upon ovulation from the contralateral ovary. It had previously been shown that the mechanical rupture of follicles in one ovary immediately after coitus did not prevent ovulation from the contralateral ovary (Friedman, 1931). Although it seemed unlikely that follicles which were subjected to injection would differ markedly in this respect from follicles which had been pricked open, it was necessary to make certain of this. The results obtained in group C of the first series showed that the unilateral intrafollicular injection of an active urine extract did not prevent ovulation from the contralateral ovary following the intravenous injection of a minimal effective dose of the same extract. In these experiments the total amount of extract received by the animal exceeded the minimal effective dose by whatever amount of the extract was absorbed from the injected follicles, and the small and variable amounts which were permitted to leak into the abdominal cavity of the rabbit by any undetected fault in the gauze blockade around the injected ovary. Although it had been

repeatedly shown that such quantities of extract are far too small to have any observable effect on the untreated ovary, it seemed advisable to extend such experiments by substituting saline for the active extracts in the intrafollicular injection.

Consequently the ripe follicles in one ovary of each of three post partum rabbits were carefully injected with saline immediately after coitus (etherisation started 5 minutes after coitus). The technique of the saline injections was identical with that used for the intrafollicular injections in groups A and D of series I. At laparotomy 24 hours later it was seen that ovulation had occurred from the untreated ovary in each of the three does. The incisions were closed in the usual fashion, and the animals permitted to live for four days longer, at which time they were sacrificed and their ovaries preserved for microscopic study. As might be expected, the corpora lutea in the untreated ovaries were almost solidly filled with lutein tissue, there being practically no central cavity. The corpora lutea in the injected ovaries, however, were not solidly filled with lutein cells, there being in each a central cavity of varying size, filled with blood.

Similar results were obtained in three animals in which an intravenous injection of a minimal effective dose of an extract of pregnancy urine was given immediately after the unilateral intrafollicular injection of saline. In no case did the operative procedures prevent ovulation from the contralateral, untreated ovary.

These experiments clearly show that if injected follicles are able to utilise gonadotropic materials more efficiently than unruptured follicles, the difference is not so great as to prevent the unruptured follicles from responding in a normal manner to the humoral changes occurring during the ten or more hours preceding ovulation, whether the ovulation is induced by coitus or by the intravenous injection of a m. e. d. of an active extract.

B. The utilisation of subminimal quantities of gonadotropic material by injected and by unruptured follicles. Despite the decisive character of the results reported in the preceding section, they did not preclude the possibility that a difference in the utilization of gonadotropic material by ruptured and unruptured follicles might become apparent when the concentration of such material was less than that present in the blood during the ten or more hours before ovulation. It had been demonstrated in earlier experiments that repeated intravenous injections of subminimal doses (less than 1 rat unit per kilo) of an active extract would produce in a post partum rabbit either enlarged cystic follicles (2 to 5 mm. diameter), clear cystic follicles with a very narrow lutein border (two to three cells deep), or partially luteinised corpora hemorrhagica, depending upon the size of each dose and the number of doses administered (Friedman, 1932b). If the follicles ruptured by injection were able to utilise the gonad-stimulating substances more efficiently than unruptured follicles, one might expect

to luteinise ruptured follicles with repeated injections of subminimal doses at a rate which would produce little or no luteinisation in ripe, unruptured follicles.

To test this possibility, the following experiments were performed. The ripe follicles in one ovary of each of eight rabbits were injected with sterile saline. The technique employed was the same as that for all previous intrafollicular injections, the amount of saline discharged from the syringe being less than 0.04 cc. per animal. Immediately following the operation, and once each day on succeeding days these eight females were given intravenous injections of an active extract according to the following schedule: rabbits 1 and 2, 0.3 m. e. d. daily for four days; number 3, 0.4 m. e. d. for four days; rabbits 4 and 5, 0.5 m. e.d. for three days; number 6, 0.7 m. e. d. for two days; and rabbits 7 and 8, 0.7 m. e. d. for three days. All of these eight animals were sacrificed six days after the intrafollicular injection of saline, and their ovaries prepared for microscopic examination. In the untreated ovaries of rabbits 1 and 2, three were several cystic follicles, 3 to 5 mm. in diameter, with no trace of luteinisation. The follicles in the injected ovary of these two rabbits were quite as large and quite as free of lutein tissue, although one of the large follicles showed a little blood in the cavity. In all the large follicles of the untreated ovaries of numbers 3, 4 and 5 there was a narrow lutein border (2 to 3 cells deep). This picture was exactly duplicated in the injected ovaries of these three animals, the lutein border being quite as narrow. In rabbit 6, the degree of luteinisation was about the same as that in numbers 3, 4, and 5, and here again there was no detectable difference between the treated and untreated ovary. In this animal, however, there were three partially luteinised follicles in the injected ovary, whereas the untreated ovary contained only one partially luteinised follicle. The degree of luteinisation found in rabbits 7 and 8 was the greatest seen in this group, the lutein border occupying fully one-third of the diameter of the structure on each side, so that the central cavity was limited to somewhat less than one-third of the diameter. Nevertheless the degree of luteinisation in the untreated follicles was equally as great as that found in the follicles in the injected ovary.

The results of these experiments, therefore, give no indication that follicles ruptured by the procedures employed in direct intrafollicular injection are more easily luteinised by subminimal doses of an active extract than are ripe unruptured follicles.

Discussion. Of all the results obtained, the most surprising were those which indicated the relative insignificance of the influence of ripe follicles on the development and functional activity of the unilateral corpora lutea. If there is any *physiological* antagonism between the hormones of the corpus luteum and of the ripe follicle such antagonism was not apparent in these experiments. Equally unexpected were the indications of the

importance of unappreciated differences in the degree of operative trauma. The operator, of course, appreciated that all operations were not consummated with the same degree of success. In some instances a clumsy hand thrust the needle through the follicular wall into the interstitial tissue. Due to such accident, and to others, there was in some cases an abnormally great amount of follicular hemorrhage. At the end of each operation, the experimenter made an attempt to evaluate the success of each of the procedures involved, and his diagnosis of the operation as a whole was recorded on the animal's history card as "poor," "fair," or, "good." In general, the character of the unilateral corpora lutea so produced tallied well with these notations on the history card. In no case did a "poor" operation produce any unilateral corpora lutea. Yet, not every "good" operation was followed by the growth of "good" corpora lutea, and in one case a "fair" operation resulted in the growth of functional lutein tissue. It is obvious, then, that the significant differences in the degree of operative trauma were not registered by the operator.

Nor was the microscopic examination of more service in detecting these differences. It is appreciated that the histological technique employed might have been inadequate to disclose the significant structural differences, and that a more suitable technique might reveal a satisfactory correlation between structure and function. This, however, seems improbable in view of the fact that some of the fully functional corpora lutea were obviously inferior (smaller, paler, cells more vacuolated) to some which were apparently without function. It must be remembered that the ovaries under consideration were removed for study at a time (12th to 14th day) when the corpora lutea of pseudopregnancy have passed the height of their functional activity. There is little doubt that the rate of regression is not identical for all corpora lutea. The chance exists, therefore, that an adequate histological examination at the height of functional activity (7th or 8th day of pseudopregnancy) would regularly distinguish the fully functional from the submaxillary functional corpora lutea.

The phrase "fully functional" is used advisedly here, because of the negative uterine response to the thread in the presence of only one corpus luteum (see group B of series I). It is not unlikely that the decidual reaction requires more lutein hormone than does the proliferative reaction. It is possible that a more sensitive criterion (e.g., inhibition of uterine motility—Reynolds and Allen, 1932) would reveal most of the unilateral corpora lutea produced by the direct injection to be submaximally functional rather than functionless.

Regardless of the degree of functional activity of most of the unilateral corpora lutea, it is significant that some of them were developed and maintained in a functional state in the absence of any humoral changes of

sufficient magnitude to be registered by the untreated follicles of the opposite ovary. If one could assume that these untreated follicles were as sensitive indicators as the ripe follicles in ovaries of an untreated postpartum rabbit, then one could safely say that the growth and maintenance of the functional unilateral corpora lutea in these experiments was effected without the secretion of a special luteinising hormone, and without the continued secretion of a *single* gonad-stimulating factor at a greatly increased rate (certainly less than the amount sufficient to duplicate the humoral condition produced by the daily injection of 0.3 m.e.d. of a urine extract).

Such statement could not be accepted, however, until it could be demonstrated that the sensitivity of the ripe follicles of the untreated ovary was unaltered either by the operative procedures involved in the intrafollicular injections, or by the growth of functional lutein tissue. From the experiments in series II it is readily apparent that the unilateral intrafollicular injection of either saline or an active extract does not prevent the normal response of the ripe follicles in the untreated ovary to those humoral changes which occur in the ten or more hours preceding ovulation. These experiments also show that the partial luteinisation of such ruptured follicles does not interfere with the luteinisation, pari passu, of the unruptured follicles in the contralateral ovary. But these experiments do not show whether or not fully functional corpora lutea render ripe follicles less responsible to gonadotropic substances. It has long been accepted that one of the functions of the corpus luteum is the inhibition of ovulation. Even granting this, the question arises as to how this "inhibition" is effected. Is it by decreasing the sensitivity of the ovarian elements to the pituitary secretions, or by decreasing the secretory activity of the pituitary?

Despite the recent work of Patel (1930) there is at hand no satisfactory, direct, quantitative evidence on these questions, but there is much indirect evidence which is relevant. It is known for example, that in most species ovulation does not occur during pregnancy, pseudo-pregnancy, or lactation; i.e., in the presence of a functional corpus luteum. In view of this, it was generally assumed that the cyclic ovarian changes also ceased, and that the follicular apparatus was suspended in a state of rest until the regression of the corpus luteum. There are enough data in the literature, however, to indicate that quite the reverse is true. According to Loeb (1911) the maturation of follicles continues in the pregnant guinea pig. Swezy and Evans (1930) report that pregnancy in the rat does not interrupt the cyclic maturation of follicles. Although the large follicles, which are developed every four or five days throughout pregancy, do not ovulate they become luteinised to form corpora lutea of about one-third the size

of those of pregnancy. In the rabbit large follicles appear in the ovaries as early as the sixth day of gestation, and from this time on an increasing number of does will accept coitus (Hammond and Marshall, 1925). Yet, insofar as nothing is known of the blood hormone content in these species during pregnancy, it is impossible to say whether or not the ovarian changes in pregnancy are brought about by larger amounts of gonad-stimulating material than are required to produce the same changes in the non-pregnant animal. Blood studies in the mare (Cole and Hart, 1931) have revealed the presence of gonadotropic material in detectable quantities during a limited period of gestation (40th to 150th days), whereas similar studies on non-pregnant mares throughout the greater part of the oestral cycle have failed to reveal such material. In this limited period of gestation large follicles appear and new corpora lutea are formed. Nevertheless, even these interesting findings in the mare are not sufficient to permit a statement as to the effect of functional lutein tissue on the sensitivity of large follicles to gonad-stimulating material.

More directly applicable to this question are the recent experiments of Snyder and Wislocki (1931). In a series of pregnant rabbits they found that there was a progressive decrease in the amount of extract required to provoke ovulation. Whereas 10 cc. or more were required to produce ovulation on the second day after mating, 2 cc. of the same extract produced ovulation on the seventh day after mating. When one considers that implantation of the rabbit embryos takes place on the eighth day after mating, it is apparent that the minimal ovulating dose of the extract in question was less at the height of activity (8th day) of the corpora lutea than during the formative period of these corpora lutea.

In general, then, it is clear that the presence of functional corpora lutea does not prevent the ovarian response to gonad-stimulating material; and particularly in the rabbit it is difficult to interpret the data at hand as indicating an inhibition of the usual gonadotropic effects by the functional activity of corpora lutea, unless there occurs in the blood of the rabbit an as yet undetected, *progressive* increase in the concentration of gonad-stimulating material in the first week of pregnancy. In view of such considerations, and the data secured in the experiments of series II, the production of unilateral corpora lutea by the direct follicular injection of active extracts seems to afford examples of the production and maintenance of functional corpora lutea in the absence of the secretion of a special luteinising hormone, or the secretion of a single gonadotropic substance at a greatly increased rate. A more definite statement than this cannot justifiably be made at this time, and must await quantitative studies on the amounts of active extract required to ovulate ripe follicles in the presence of functional, unilateral corpora lutea.

SUMMARY

1. By the direct intrafollicular injection of extracts of urine of pregnancy it is possible to produce functional corpora lutea unilaterally.

2. The presence of the contralateral, untreated ovary appears to have little or no influence on the development of functional lutein tissue by this means. Moreover, the presence of the ripe follicles in the untreated ovary does not prevent the manifestation of functional activity by the unilateral corpora lutea. If there exists a *physiological* antagonism between the secretions of ripe follicles and those of corpora lutea, such antagonism was not evident in these experiments.

3. Mechanically ruptured follicles are not more easily luteinised than are ripe, unruptured follicles. The direct injection of the ripe follicles of one ovary does not modify the response of the untreated, ripe follicles of the opposite ovary to those humoral changes which occur in the ten or more hours preceding ovulation, or to those humoral changes produced by the repeated intravenous injection of submaximal doses of active extracts.

4. The production and maintenance of functional corpora lutea unilaterally in the absence of any discernible change in the ripe follicles of the untreated, contralateral ovary, appears to afford examples of the growth and maintenance of functional lutein tissue without the secretion of a special luteinising hormone by the pituitary (or any other gland), or the secretion of a single gonadotropic hormone at a greatly increased rate.

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CIRCULATORY ADJUSTMENTS TO MODERATE EXERCISE IN NORMAL INDIVIDUALS, WITH PARTICULAR REFERENCE TO THE INTERRELATION BETWEEN THE VELOCITY AND VOLUME OF THE BLOOD FLOW

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Received for publication May 6, 1932

Studies reported on the hemodynamic changes in man during exercise have dealt mainly with changes in the volume flow of blood, in the pulse rate and blood pressure, with the physico-chemical properties of the blood and with alterations in the respiratory functions of the organism. The present study is concerned with the effect of exercise on several measurable factors of the circulatory hemodynamics in normal individuals. In particular, the relation of changes in the blood velocity to alterations in the volume flow of blood were investigated. It has generally been assumed that variations in the velocity of blood flow roughly parallel the changes in the total volume of the blood flow in normal individuals. The blood velocity or the circulation rate may be defined as the shortest time in which a particle of blood or injected material passes from one arbitrarily chosen site in the vascular system to another. This circulatory pathway is usually from one of the superficial veins, most commonly in the arm, through the pulmonary circulation to some arterial or venous location, as in the brachial artery, the carotid sinus, the minute facial vessels or the opposite cubital vein. The total blood flow is, of course, the amount of blood pumped by one of the ventricles of the heart in a given period of time. If the total cross-sectional diameter of the vascular bed remains unchanged, changes in the volume of blood flow will produce parallel changes in the velocity. If, however, the cross sectional area of the peripheral capillary bed is altered these parallel changes will not occur. For instance, if the cross-sectional area of the total pulmonary vascular bed is doubled by the opening up of new capillaries and the engorgement of those already open, then the circulation time will be doubled although the total volume of blood flow is unchanged. Therefore, if the circulation rate and volume of blood flow are simultaneously determined in the same individual and are correlated with changes in the arterial and venous blood pressures, with the respiratory exchange of gases, the vital capacity of the lungs, and the

heart rate, significant information regarding the changes which take place in the pulmonary circulation during exercise may be obtained.

METHODS. The experiments were performed on 5 healthy male subjects, all physicians or medical students ranging in age from 24 to 32 years. None were in a state of "training" at the time of the experiments, although one (J. H.) had been athletic in college, and both he and a second (L. E.) were accustomed to frequent, though intermittent, exercise chiefly in the form of squash racquets or tennis. The exercise was in each case performed on a bicycle ergometer equipped with a Prony brake. An attempt was made to have the work done per minute kept constant for each subject throughout the experiment and to have each subject perform a standard exercise of moderate degree. We were not completely successful in this last endeavor since the heat equivalent of the work done actually varied in the different subjects from 0.666 to 0.965 kgm. Cal. per minute. In no instance did the subjects complain of undue fatigue at the end of the exercise period. The experiments were all performed from one to three hours after the last meal. On each subject the experiments were performed in 2 stages. In the first part of the experiment the cardiac output and the blood velocity measurements were made, both at rest and during exercise. In the second part, the remaining measurements were made on a subsequent day under conditions as nearly identical to those of the first day as possible. The room temperature during all experiments was kept at from 23 to 25 degrees Centigrade.

The cardiac output was determined by the Fick principle, using the technique designed for exercise described by Bock, Dill and Talbott (1928). We believe that this method is the most suitable one at present available for determining the cardiac output *during exercise*, and that although the absolute results may be open to some question, it provides an accurate relative indication of changes in blood flow. Individual dissociation curves for carbon dioxide were not determined, as this seemed unnecessary with the comparatively low grades of exercise employed.

The blood velocity of circulation time was measured by the use of sodium cyanide according to the technique devised by Robb and Weiss (1932b). About 0.05 to 0.13 mgm. per kgm. of body weight of the cyanide in 2 per cent solution was injected into the cubital vein and the appearance of its stimulating action on the respiration timed. This gave a measure of the velocity of blood flow from the cubital vein to the right heart, through the lungs, and to the carotid sinus, where the drug acts by immediately increasing the rate and depth of respiration (Heymans et al., 1931). In an extensive series of experiments the originators of this method have compared it with other methods for determining the circulation rate and have found it to be accurate. The method recommends itself particularly by its extreme simplicity, by the absence of any unpleasant side-effects,

by the very transitory nature of its direct effects and by the fact that it can be repeated frequently at short intervals. It is also entirely objective and the circulation time is registered graphically. In the present study the circulation time was graphically recorded on a kymograph, by means of a time marker, an electric signal depicting the moment of injection, and a pneumographic tracing of the respiration. All determinations of the circulation rate were checked by repetition and the results always checked within a second.

The arterial blood pressure was determined by a mercury sphygmomanom-

TABLE 1

The effect of moderate exercise on the cardiac minute and stroke volume outputs, the heart rate, the circulation rate, and the respiratory exchange in 5 normal individuals

SUBJECT	REMARKS	HEART RATE	RESPIRATORY RATE	CO_2 TENSION			RESPIRATORY MINUTE VOLUME	CO_2 OUTPUT	CARDIAC OUTPUT PER MINUTE	CARDIAC OUTPUT PER BEAT	OXYGEN CONSUMPTION	RESPIRATORY QUOTIENT	CIRCULATION RATE	HEAT EQUIVALENT OF WORK DONE	EFFICIENCY
				Virtual venous	Alveolar	Difference									
				mm. Hg	*mm.* Hg	*mm.* Hg	*liters*	*cc. per minute*	*liters*	*cc.*	*cc. per minute*		*seconds*	*calories per minute*	*per cent*
J. H.	Sitting	80	14	43.2	35.4	7.8	9.3	284	7.6	95	363	0.77	19		
	Bicycling	114	20	58.2	44.2	14.0	26.7	1,195	19.4	170	1,310	0.91	11	0.873	18.7
M. K.	Sitting	93	18	49.5	41.3	8.2	8.3	283	7.8	82	313	0.90	13		
	Bicycling	140	36	68.2	50.2	18.0	29.9	1,442	22.7	162	1,486	0.97	8.5	0.915	15.8
B. M.	Sitting	98	22	47.6	39.1	8.5	7.2	220	5.9	64	277	0.79	15		
	Bicycling	136	32	62.0	45.0	17.0	22.8	962	15.0	110	1,040	0.92	11.5	0.696	18.5
L. E.	Sitting	88	14	47.8	39.5	8.3	9.4	328	8.6	98	363	0.88	17		
	Bicycling	137	21	64.0	46.7	17.3	28.3	1,287	19.8	146	1,335	0.96	8.5	0.965	20.0
S. W.	Sitting	100	17	51.7	42.2	9.5	8.5	265	6.8	68	311	0.84	17		
	Bicycling	139	24	68.0	50.0	18.0	28.0	1,232	19.3	138	1,274	0.96	10	0.844	17.6

eter. The venous blood pressure was measured by the Eyster (1929) modification of the indirect method of Hooker, the measurement being made over a superficial dorsal vein of the hand, the hand and arm being supported extended at a level a few centimeters below that of the right auricle. The pulse rate was counted at the wrist or over the carotid artery. The vital capacity was obtained by a Collins spirometer.

RESULTS. *Cardiac output, heart rate, total ventilation, respiratory quotient, and oxygen consumption.* The changes which these factors undergo when the subject shifts from a resting sitting position on the bicycle to a state of exercise entailing an oxygen consumption of 1.0 to 1.5 liters per minute

are given in table 1, and some of them are graphically depicted in chart 1. They are in close agreement with those reported and discussed by Bock, Van Caulaert, Dill and their associates (1928). In the present series of experiments the resting pulse rates of the subjects tended to be rather higher than one would expect. This was probably due to the psychic stimulation occasioned by the strangeness of the technical procedures.

The velocity of blood flow (circulation rate). The effect of exercise on the circulation time of the 5 subjects is shown in table 1 and chart 1. The circulation rates at rest varied from 13 to 19 seconds in the different subjects, and these figures are in agreement with the normal values found by Robb and Weiss (1932b), using the same method. It will be seen that in every case the velocity of blood flow increased with exercise (i.e., the circu-

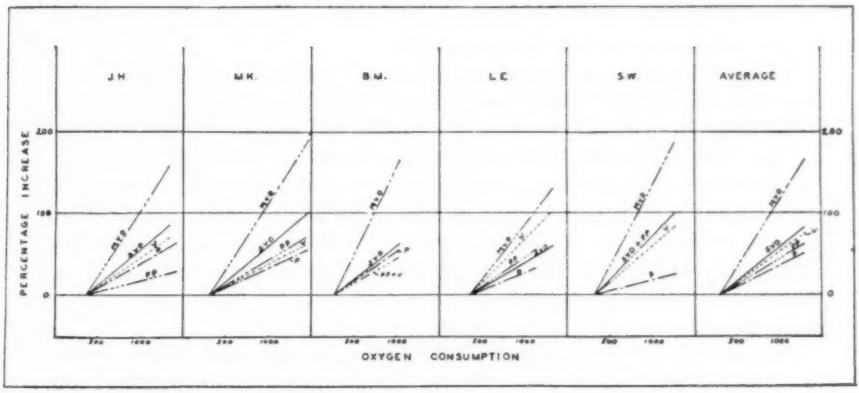

Chart 1. The percentage increase during exercise in the cardiac minute and stroke volume outputs (*M V O* and *S V O*), the velocity of the blood flow (*V*), the arterial pulse pressure (*P P*), and the pulse rate (*P*) in 5 normal individuals.

lation time was shortened). This increase in velocity of blood flow was not, however, comparable in degree to the increase in cardiac output, since the velocity increased from 50 to 100 per cent, whereas the volume of blood flow increased from 130 to 190 per cent over the resting value.

The circulation time as determined in the present experiments is essentially a measure of the velocity of venous blood flow from the cubital vein to the right auricle plus the velocity of blood flow through the pulmonary circulation. In normal resting persons with the same method, the circulation time in the vein to the right auricle averages about 5 seconds and the crude pulmonary circulation time is 11 seconds (Robb and Weiss, 1932b). In other words, the venous component of the total determined circulation time is about 30 per cent of the total. In the present experi-

ments it was impossible to determine directly the venous and the pulmonary elements of the velocity measurements during exercise. It is theoretically possible that during exercise such as was employed, which entails muscular activity chiefly in the legs, the velocity of venous blood flow in the comparatively inactive arms is either unchanged or even slightly slower than at rest. If this assumption is made and the results recalculated on this basis then the proportionate increase in the velocity of blood was still less in 4 of the 5 cases than the increase in the cardiac output. However, it is possible to obtain indirect evidence that the velocity of blood flow in the arm during such exercise is actually increased. If a determination of the oxygen content of blood drawn from the cubital vein both at rest and during exercise is made, an increased oxygen saturation of this venous blood during exercise is clear evidence of an increase in the blood flow, since the metabolism of the arm during the exercise is either unchanged or somewhat increased.

This experiment was performed on 5 normal subjects and the results are recorded in table 2. Blood samples were drawn under oil without stasis from the outstretched arm after the subject had remained quietly sitting on the bicycle 10 minutes and again during exercise at the end of 15 minutes of bicycling. The amount of work done was of the same order of magnitude as in the other experiments of this study and the room temperature was maintained close to 25°C. throughout, since it is known (Dill et al., 1931) that variations in the external temperature influence the blood flow in exercise. The oxygen contents and capacities of the blood samples were determined.

In every one of the 5 subjects the degree of oxygen unsaturation during exercise was less than at rest, although the amount of the difference varied in the different individuals. This, then, is substantial evidence in favor of an increased blood flow through the arm during bicycling with the external temperature at about 25 degrees.

Since the size and degree of distention of the peripheral veins during exercise were in no case perceptibly greater than at rest, and in 2 instances (J. M. and A. H.) the veins were clearly smaller, it is safe to assume that the velocity of blood flow in the cubital vein was definitely increased during exercise, although the quantitative extent of this increase could not be definitely computed.

Although it is obviously impossible to draw any definite quantitative conclusions as to the relationship of the increased velocity and total volume of blood flow during moderate exercise, one can say with assurance that both invariably increase in the peripheral and the pulmonary circulations, but in the pulmonary circuit the increase in velocity is of less extent than in the total volume of blood flow.

Arterial blood pressure. (Table 3, chart 1.) The blood pressure of the

5 subjects responded in very different degrees to the exercise, but all showed a considerable rise in systolic and a very slight increase in diastolic pressure, with a consequent increase in the pulse pressure. Similar findings regarding the effect of exercise on the arterial blood pressure have been reported before (Gillespie et al., 1925; Bock, Van Caulaert et al., 1928; Paterson, 1928). The increase in pulse pressure is probably due largely to the increase in the stroke volume output of the heart. The great variability in the blood pressure response of different individuals to exercise, however, is an indication that the adaptive mechanisms in the peripheral circulation are also of great importance in determining the extent of increase in the pulse pressure.

TABLE 2

The oxygen content and capacity of blood drawn from the cubital vein at rest and during exercise

NAME	REMARKS	OXYGEN CAPACITY	OXYGEN CONTENT	PER CENT SATURATION	DIFFERENCE	HEAT EQUIVALENT OF WORK DONE
		volumes per cent	*volumes per cent*		*per cent*	*calorie per minute*
L. E.	Sitting	20.03	17.80	89		
	Bicycling		18.71	94	+5	0.848
S. W.	Sitting	20.50	12.11	59		
	Bicycling	21.45	13.33	62	+3	0.847
G. R.	Sitting	19.58	11.44	58		
	Bicycling	20.19	13.15	65	+7	0.868
J. M.	Sitting	19.95	10.85	54		
	Bicycling		17.46	88	+34	0.906
A. H.	Sitting	20.40	6.24	31		
	Bicycling		14.30	70	+39	0.960

Venous blood pressure. (Table 3.) In every case the venous blood pressure rose during the exercise and was either maintained at the increased level until the exercise ended or fell slightly as the work continued. An increase in venous pressure during the performance of exercise has been described before (Hooker, 1911; Schneider et al., 1918; White, 1924; White and Moore, 1925). So far as they go, our findings are essentially in accord with those previously reported. White (1924, 1925) has published results tending to show that during the maintenance of dynamic exercise of a moderate degree of severity there may be a tendency for the venous pressure to fall from its initial high point toward a level that approaches the normal, and with the cessation of the exercise the pressure immediately

becomes normal or subnormal. This finding he ascribes to increased ease of filling of the right side of the heart due probably to a decreased diastolic tone of the heart occurring during the course of the exercise. The increase

TABLE 3

The effect of moderate exercise on the heart rate, the arterial and venous blood pressures, and the vital capacity of the lungs in 5 normal individuals

SUBJECT	REMARKS	TIME FROM START OF EXERCISE	HEART RATE	ARTERIAL BLOOD PRESSURE		VENOUS PRESSURE	VITAL CAPACITY OF LUNGS	HEAT EQUIVALENT OF WORK DONE
				Systolic	Diastolic			
		minutes		*mm. Hg*	*mm. Hg*	*cm. H_2O*	*cc.*	*calorie per minute*
J. H.	Sitting	0	60	110	65	6	6,100	
	Bicycling	5	100	140	80	12	5,900	
	Bicycling	10	100	138	78	11	5,950	0.954
	Sitting (5 minutes after stopping)		66	112	70	8	6,200	
M. K.	Sitting	0	82	113	76	3	5,600	
	Bicycling	2	128	144	80			
	Bicycling	7		175	85	5		
	Bicycling	10	126	175	86	3	5,600	0.906
	Sitting (3 minutes after stopping)		96	130	76	3		
B. M.	Sitting	0	72	104	68	4	3,800	
	Bicycling	5	112	130	75		3,750	
	Bicycling	9	116	132	75	8		0.666
	Sitting	0	86	102	78	4	3,850	
	Bicycling	4	130	110	76		3,750	
	Bicycling	10	130	126	80	12	3,800	0.924
	Sitting (2 minutes after stopping)		100	100	76	10		
	Sitting (12 minutes after stopping)		84			5		
L. E.	Sitting	0	84	110	65	1	4,900	
	Bicycling	5	112			4	4,800	
	Bicycling	10	112	150	80	4	4,800	0.833
S. W.	Sitting	0	100	124	82	9	5,100	
	Bicycling	4	126	185	90	13	5,000	
	Bicycling	8	126	180	88	13	5,200	0.895

in venous pressure is evidence of the increased venous backflow to the heart which is produced by the pumping action of the active skeletal musculature and of the diaphragm, by the suction effect of the increased respiratory movements, possibly by a change in the diastolic cardiac tone and, to a

slight extent, by the dilatation of many of the peripheral arterioles and capillaries. The extent to which the pressure rises in any individual case is the resultant of the interaction of these various factors.

Vital capacity of the lungs. (Table 3.) The vital capacity was essentially unchanged during exercise in all the subjects.

Discussion. The changes occurring during exercise, as determined by the various measurements described above, may be correlated by a theoretical analysis of some of the circulatory adjustments which take place in the cardiovascular system. Although the responses of different individuals to a given exercise are qualitatively similar, the quantitative extent of their adjustments is very variable.

With the beginning of exercise the venous backflow to the heart is increased and the venous pressure tends to be raised. Probably as a result of this increased venous return, the heart is stimulated to greater activity and its output is increased.

The fact that during exercise the degree of increase in the velocity of blood flow is consistently less than in the volume of blood flow through the lungs, suggests that during muscular exercise there is, in addition to the increase in the velocity of blood flow through the pulmonary circuit, an increase in the cross-sectional area of the pulmonary vascular pathways. The increase, however, is not marked after such exercise and the main change in the volume of blood flow is due to change in the velocity. Thus the dynamics of the pulmonary circulation during exercise of normal subjects is essentially different from that postulated (Weiss, 1931) and found by Robb and Weiss (1932a) in cardiac patients with circulatory failure at rest. This difference between the behavior of the pulmonary circulation during exercise in normal subjects and in resting patients with circulatory failure is particularly significant as it suggests the possibility that the mechanism of dyspnea in these two states of the body is different. The rôle of the pulmonary circulation and its influence on the physiological state of the alveoli and the lungs as a whole in patients with circulatory failure was discussed, and the mechanism by which an increased pressure in the pulmonary vein, capillaries and arterioles leads to functional emphysema of the lungs was described by these authors. In normal subjects during exercise a similar mechanism in the lungs apparently does not exist.

The experimental evidence that during exercise the cross-sectional diameter of the pulmonary capillary bed increases does not throw light on the question of whether such an increase in the total cross sectional area is due to an increase in the number of open capillaries, or to an increased engorgement of the previously open capillaries. During exercise, however, the blood flow through the visible human capillaries is not, in our experience, associated with engorgement of the individual capillaries and evidence is available that the lung contains reserve capillaries collapsed at rest

(Toyama, 1925; Wearn et al., 1926). The concept that closed capillaries open during exercise appears therefore to be the most probable explanation of the observations reported.

SUMMARY AND CONCLUSIONS

1. In 5 normal individuals estimations were made of the cardiac minute and stroke volume outputs, the respiratory exchange, the velocity of blood flow, the cardiac rate, the arterial and venous blood pressures and the vital capacity of the lungs both at rest and during the performance of a moderate exercise.

2. The velocity of blood flow from the cubital vein through the pulmonary vessels to the carotid artery is increased during exercise to a less extent than the minute volume of the heart.

3. During exercise the systolic arterial blood pressure is definitely increased but there is little or no increase in the diastolic pressure. The venous pressure is increased.

4. The vital capacity of the lungs does not change significantly.

5. The significance of these findings is discussed in relation to the changes in the hemodynamics during exercise.

I take great pleasure in acknowledging my indebtedness to Dr. Soma Weiss for his assistance and advice in this research and to Miss Rose Shore for her technical assistance.

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FACTORS INFLUENCING ANEMIA DEVELOPMENT IN YOUNG RATS

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Received for publication May 10, 1932

Nutritional anemia in young rats is produced in most laboratories by feeding a milk ration from the time of weaning or before. In spite of every precaution that the young shall never obtain any of the mother's dry ration, and shall receive only mother's milk and later cow's milk, the hemoglobin content of the blood of young rats at weaning is far from uniform. The variation is but slight among those of the same sex in any one litter but there is a difference between sexes and between litters of the same age and heritage which is not easily explained.

Data accumulated in this laboratory on all rats used for anemia work during the past two years have been analyzed in an effort to determine what factors, if any, are consistently responsible for the variations noted. Complete records of 570 young rats from 73 litters plus blood histories of 42 of the mothers during pregnancy and lactation afford information which should throw some light on the problem. If similar variations have been been noted by other investigators, such a study may aid in the standardization of technique to be used in further nutritional anemia work. Details regarding our procedure of bleeding the rats and making hemoglobin determinations are given in a previous paper (1). All hemoglobin figures or averages given in the present paper are based on the average hemoglobin value for all rats of the same sex in any given litter. This procedure avoids unnecessarily detailed work and gives equal weight to the figures from each litter regardless of the number in the litter and the distribution of the sexes.

The differences in the initial hemoglobin figures of the young at weaning seem to be chiefly responsible for differences in the time required to produce a severe anemia. Correlations have, therefore, been attempted between the hemoglobin content of the blood of young rats at weaning and several different factors which might conceivably have some influence on this blood story. These considerations are previous diet of mother, parity of the mother, blood history of the mother during pregnancy and lactation, size of the litter, age and weight of the young at first hemoglobin determination, method of caging and sex differences.

1. *Previous diet of the mother.* The stock ration used in this colony consists of:

	per cent
Dried whole milk	20
Rolled oats	10
Peanut meal	12
Yellow corn (ground)	10
Dried celery tops	3
Dried whole wheat bread	42
Wheat germ	2
Yeast	0.5
Salt	0.5

Fresh greens are fed about five times a week. The adequacy of this ration has been demonstrated by eight years' successful use indicated by a high normal growth curve and a splendid reproductive record (2). When the pregnant females are isolated, two or three days before parturition, they are given milk ad libitum and one teaspoonful of wheat germ daily as supplements to the regular stock ration. These and other supplements which have been made in certain cases are always discontinued when the litter is 14 days old to prevent the young obtaining any possible supplementary food. In a previous paper (3) it was reported that neither a mineral supplement in the form of soluble iron, copper and manganese

TABLE 1

Correlation of mother's ration with hemoglobin of young at weaning

	NUMBER OF LITTERS	HEMOGLOBIN, GRAMS PER 100 CC. OF BLOOD	
		Males	Females
Stock ration without supplement	53	8.0	8.9
Stock ration and 0.5 mgm. Fe, 0.5 mgm. Cu and 0.1 mgm. Mn daily	10	8.8	9.3
Stock ration and yeast extract (Savita) 0.5 mgm. daily	7	9.1	10.0

salts, nor an addition of vitamin B complex in the form of a yeast extract exerted any apparent influence in the prevention of the anemia of pregnancy previously noted when animals were fed on the regulation stock ration. In spite of these negative findings it was still possible that the mineral or vitamin supplements to the mothers' ration might increase the resistance of the young to nutritional anemia. Sure and Kik (4) found the concentration of hemoglobin in nursing young to be definitely affected by the diet of the mother. The results of the present survey in this respect are given in table 1.

Although the variation is but slight *the hemoglobin content of the blood of young rats at weaning appears to be influenced to some extent by the previous*

diet of the mother. Experience of other laboratories (5) in which hemoglobin concentrations as low as 2.0 to 3.0 grams per 100 cc. of blood were obtained in rats as early as 8 days after weaning may be due to a stock ration less rich in either the mineral or vitamin B complex than is our original stock ration. No extensive observations have been made in this laboratory on any other type of stock ration.

TABLE 2

Correlation of parity of the mother with hemoglobin of young at weaning

PARITY OF THE MOTHER	NUMBER OF LITTERS	HEMOGLOBIN, GRAMS PER 100 CC. OF BLOOD	
		Males	Females
1st litter	17	7.5	8.3
2nd litter	20	7.9	8.5
3rd litter	12	8.5	9.5
4th–6th litters	7	8.9	9.3

TABLE 3

Hemoglobin of young rats at weaning contrasting several successive litters from the same mothers

RAT NUMBER	BIRTH OF LITTER DATE	NUMBER IN LITTER	AGE WHEN SEGREGATED	HEMOGLOBIN, GRAMS PER 100 CC.		ANEMIA PRODUCED, NUMBER OF WEEKS		RATION OF MOTHER
				Males	Females	Males	Females	
			days					
3339	12/17/29	6	35	6.3(4)	6.3(2)	1.0	1.0	Stock
	2/23/30	9	29	8.1(4)	8.5(5)	4.0	5.0	Stock and Fe, Cu, Mn
	5/ 3/30	11	31	11.1(3)	11.7(4)	5.3	5.2	Stock and Fe, Cu, Mn
	7/27/30	10	33	12.4(6)	12.6(4)	6.0	5.3	Stock and Yeast Extract
3345	9/ 9/29	12	35	5.3(1)	6.4(6)	1.0	1.3	Stock
	11/21/29	11	40	5.4(3)	5.8(4)	1.0	1.0	Stock
	2/ 2/30	12	30	7.3(5)	8.0(6)	4.0	4.0	Stock and Fe, Cu, Mn
	6/17/30	11	28	10.0(6)	11.0(5)	3.5	4.8	Stock and Yeast Extract
	9/ 4/30	5	32	12.3(5)		5.6		Stock

Note: Figures in parentheses indicate the number of each sex used in the experiments.

2. *Parity of the mother.* Litters for anemia experiments have been used as they became available in the breeding colony with no special attention to the parity of the mother. It was noted, however, that first litters seemed to show a more anemic condition at weaning than subsequent litters. A survey from this standpoint has, therefore, been made of all litters used in the present study. The 56 litters in which no dietary change

had been made during the reproductive cycle have been used in arriving at the averages (table 2). These averages afford some evidence that parity of the mother may influence the blood picture of the young but these differences alone would not be significant except for more specific data which happen to be available. In two instances several successive litters from the same mothers were used for anemia work. Hemoglobin values of the young of each litter may, therefore, be compared.

It will be noted that the age when the first hemoglobin determinations were made varies somewhat but there is abundant evidence in our records to indicate that the few days' difference here could not in any way be

TABLE 4

Correlation of hemoglobin level of mother's blood at parturition with that of young at weaning

LOWEST HEMOGLOBIN VALUE OF MOTHER'S BLOOD WITHIN A WEEK OF PARTURITION, GRAMS PER 100 CC. OF BLOOD	NUMBER OF LITTERS	HEMOGLOBIN, GRAMS PER 100 CC. OF BLOOD	
		Males	Females
grams			
15 or above	7	8.5	9.2
13–15	17	8.0	8.7
11–13	15	8.4	9.2
Below 11	3	6.8	6.8

TABLE 5

Correlation of number in litter with hemoglobin of young at weaning

NUMBER OF RATS IN A LITTER	NUMBER OF LITTERS	HEMOGLOBIN, GRAMS PER 100 CC. OF BLOOD	
		Males	Females
4–6	14	8.3	9.0
7–8	24	8.0	8.8
9–10	10	8.3	8.7
11–13	6	7.7	8.4

responsible for the wide differences in hemoglobin figures obtained. To be sure the picture is also complicated by the mineral and vitamin B supplements made to the mother's ration and may to some extent be interpreted in support of the first factor discussed in this paper. However, the *progressive nature of the differences noted in the hemoglobin values for the successive litters from the same mother would seem to be significant.* It is regretted that more extensive data of this type are not available. Other laboratories may be able to contribute further information along this line.

3. *Blood history of mother during pregnancy and lactation.* A study on

anemia in pregnancy recently made in this laboratory (3) affords data on the blood history of the mothers of 42 of the litters used in the present survey. Hemoglobin figures are available at weekly intervals throughout the gestation and lactation periods. The mothers show a significant loss in hemoglobin concentration during gestation with a minimum value just after parturition in most cases, occasionally just before. The 42 litters have been grouped according to the minimum hemoglobin concentration of the mothers' blood within a week of parturition.

There is little, if any, correlation shown in these figures unless the average figure for three litters in the last group may be considered significant. One must conclude that the *hemoglobin concentration of the mother's blood does not show any striking influence on that of the young unless the mother shows a severe degree of anemia.* Such a condition would logically predispose the young to anemia because of an inadequate storage of iron.

4. *Size of the litter.* It is logical to suppose that the more young a mother must nourish either pre- or postnatally the greater the drain on her system and the greater the chances for the young to be malnourished. To apply this theory to the blood story in the present survey all litters (54) fed on stock ration free from complicating dietary influences are grouped according to the number of young in the litter (table 5).

Much to our surprise there *appears to be little, if any, correlation between the number of rats in a litter* and *the hemoglobin value of their blood* at weaning.

5. *Age and weight of young when first hemoglobin determinations are made.* All conditions of experimental procedure have been kept as uniform as possible throughout the past three years. Rats are from a stock colony which has been practically inbred for 18 generations. Mother rats are always segregated from their litters at 21 days, and first hemoglobin determinations on young scheduled for an anemia experiment are made between the 26th and 36th day at which time the rats are separated into single cages. The body weights vary between 40 and 60 grams at this age. Rats of exactly the same age and weight may show as wide differences in hemoglobin values as those showing the maximum variation in age and weight within the above limits. Thus *no striking correlation seems to exist between the exact age or body weight of the young rats* and the *hemoglobin content of the blood.* During the first few weeks of rapid growth a normal rat on an adequate ration rapidly brings its hemoglobin up to a normal concentration (3) but on a milk ration there appears to be a period when but slight change occurs followed by a reduction of hemoglobin as the iron reserves are exhausted.

6. *Method of caging.* Several laboratories have recently emphasized the necessity of avoiding mental contamination from cages used for anemic rats. Nevans and Shaw (6) call attention to the material of which the cages are composed and access of the rat to fecal material. In this labora-

tory the cages used for mother rats with young litters have ¼ inch mesh which allows some of the feces to remain in the cages. While this source of error is fully recognized it has been a constant factor in all the work

TABLE 6

Comparison of anemia development in males and females

NUMBER OF ANIMALS AND PERIOD OF OBSERVATION	HEMOGLOBIN AVERAGES, GRAMS PER 100 CC. OF BLOOD		AVERAGE NUMBER OF WEEKS ON MILK RATION		NUMBER OF LITTERS IN WHICH AVERAGE HEMOGLOBIN VALUES SHOWED		
	Males	Females	Males	Females	♀ > ♂	♀ = ♂ ± 2%	♀ < ♂
Total 73 litters, 283♂, 291♀:							
At weaning, average	8.2	9.0			58 (80%)	8 (11%)	7 (9%)
Group I. 42 litters, 154♂, 158♀:							
At weaning, average	7.9	8.5			32	6	4
Range	(5.3 –13.1)	(5.9 –13.7)			(76%)	(14%)	(10%)
End of period on milk, Average	4.1	4.8			33	6	3
Range	(3.0 –6.2)	(3.5 –6.7)			(79%)	(14%)	(7%)
Time constant for all rats in one litter, Average			3.7	3.7			
Range			(1–12)	(1–12)			
Group II. 31 litters, 129♂, 133♀:							
At weaning, average	8.6	9.5			26	2	3
Range	(6.3 –12.6)	(6.5 –13.7)			(84%)	(6%)	(10%)
End of period on milk Average	3.5	3.6					
Range*	(3.0 –3.9)	(3.1 –4.0)					
Time required for anemia to develop, average			5.2	6.3	26	3	2
Range			(2.0 –8.6)	(2.7 –13)	(84%)	(10%)	(6%)

* Hemoglobin below 4.0 per 100 cc. for each individual rat.

Note: The hemoglobin values in 10 females of group II failed to decline to the desired level within 10–12 weeks on the milk ration and are, therefore, omitted from the above averages. In no case was a similar delay noted in males.

reported and could scarcely have been responsible for the wide variations in hemoglobin values noted in different litters considering that the values within any one litter are relatively uniform. When the rats are segregated

for nutritional anemia experiments they are placed in individual galvanized wire cages with raised bottoms of $\frac{3}{8}$ inch mesh. All feces drop through this mesh readily.

Since the chief consideration in this discussion thus far has involved the hemoglobin titre in young rats at the time the experiments are started the problem of the individual cages is irrelevant. These cages, however, should enter into consideration in relation to the declining hemoglobin values in rats during the preliminary experimental period on a milk ration. During the latter part of 1931 it was noted that the time required for reduction of hemoglobin values to 4.0 grams per 100 cc. of blood or below required longer than formerly. Some old cages which were in use at that time were suspected of being the source of supplementary minerals. A group of freshly regalvanized cages proved successful in solving this difficulty. In another instance the crowded condition of the laboratory forced us to put a litter of young together in a large cage with $\frac{1}{2}$ inch mesh bottom for two weeks of the preliminary period on milk instead of segregating them in individual cages as was customary. Instead of declining values, rapid hemoglobin synthesis occurred resulting in normal values in two weeks. In this case a rusty metal drinking cup had been used inadvertently in place of the usual glass or porcelain. Faulty caging facilities can easily vitiate all other results, as has been emphasized by other investigators.

7. *Influence of sex.* Our attention was attracted to a sex difference by the hemoglobin records of male and female litter mates which are customarily recorded on one page. Invariably the addition of mineral supplements had to be delayed longer for the females than the males. This phenomenon has proved to be the most interesting of all the factors considered in this survey. The greater resistance to anemia among females is evident no matter how the data are manipulated.

In our earlier work all of the young from one litter remained on the milk ration the same length of time and were given the mineral supplement when some or most of them had developed a severe anemia. The first 42 litters designated as group I, have therefore been studied relative to the comparative hemoglobin titre in males and females in each litter after a constant number of weeks on the milk ration. The second group of 31 litters in which a definite severe degree of anemia was allowed to develop in each rat before any supplement was added regardless of the time involved has been designated as group II. In this group comparison is made of the time required for the hemoglobin values to decline to below 4.0 grams per 100 cc. of blood.

The blood of *female rats* not only *shows a higher hemoglobin concentration at weaning* but *females require longer to develop a severe anemia* of a definite degree *than do male rats* from the same litter. In some instances this

delay seems to be due to a slower rate of decline in hemoglobin in females but from the general averages it would appear that the chief difference is in the initial hemoglobin values. The difference in the growth impulse between the two sexes might also be suggested as a possible factor affecting hemoglobin synthesis. A comparison has, therefore, been made between the rate of gain in weight versus the rate of loss of hemoglobin but there is no direct or inverse correlation apparent. It may, therefore, be concluded either that the female is endowed with a better prenatal storage of iron or that she uses the iron which is available from endogenous or exogenous sources more efficiently than does the male.

SUMMARY

1. An attempt has been made to correlate the variations noted in the hemoglobin content of the blood of young rats at weaning with several factors which might conceivably have some bearing on the problem.

2. Hemoglobin values in young rats at weaning show slight but significant correlation with the ration of the mother during pregnancy and the parity of the mother.

3. No significant correlation is apparent between the hemoglobin content of mother's blood at time of parturition and that of the young at weaning.

4. Neither age and weight of young at weaning nor size of the litter show any apparent influence on blood hemoglobin values at weaning.

5. Metal contamination from cages and food cups used may seriously interfere with the experimental production of nutritional anemia in young rats. Precaution should also be taken to have raised cage bottoms of sufficiently large mesh ($\frac{3}{8}$–$\frac{1}{2}$ inch) so that the rats do not have access to feces.

6. The sex difference is most interesting because of a consistently higher hemoglobin content of the blood of females than males and a correspondingly longer period required for severe anemia to develop.

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STUDIES IN THE MOTOR ACTIVITY OF THE LARGE INTESTINE

IV. Response to Autonomic Drugs

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Received for publication May 19, 1932

It was found in earlier work (1) that a reciprocal relationship existed between two types of activity observed in the dog's colon. The wave-like contractions characteristic of the proximal colon were found to alternate with rhythmic pulsations, which were usually seen most marked in the anal sphincter region.

Assuming that the innervation of the sphincters is such that the thoracico-lumbar fibers are essentially motor, we were interested in seeing whether, in response to sympathetic drugs such as adrenalin, we could elicit contractions of the sphincter region, along with the rhythmic pulsations throughout the colon usually associated with sphincter activity in spontaneous motility.

It had been observed that rhythmic pulsations of the distal colon could be set up by suitable mechanical or electrical stimulations of the sphincter region (2). In this, as well as in similar activity originating spontaneously, usually the entire distal colon responded as a unit, and occasionally the whole colon.

It was expected, then, that adrenalin would activate the sphincter region, and it was hoped that the response of the rest of the colon might throw some light on the origin of the rhythmic pulsations.

Methods. In the unanesthetized dog, we used both the six-balloon technique (1) and the plunger-balloon technique for longitudinal and circular activity (3), (4) previously described. The control conditions were exactly similar, with either technique, to those described in previous work. All injections were made intravenously, the crural vein being usually selected. Control injections of physiological salt solutions were given. Adequate time was allowed to elapse (1 to 2 hours) for recovery from the effects of inserting the apparatus.

In anesthetized dogs and cats the plunger-balloon apparatus was used exclusively. The belly was opened by a midline incision from the xiphoid

[1] Donnelley Fellow in Physiology.

process to the symphysis pubis, and the walls were retracted and supported by tying to uprights attached to the animal board, so as to form a deep trough in which the viscera lay exposed. Ringer's solution at body temperature was passed through the open belly by means of a pump so arranged as to re-circulate the fluid, keeping the volume in the abdomen always constant. Temperature of the immersion fluid was kept constant within about 0.2°C. by passing the fluid in the pumping system through a series of coils immersed in a thermostatically controlled constant temperature bath before delivery into the abdomen. The total volume of the fluid used in this system was usually about 1500 cc., about one-half of which represents the volume of the pump, tubes, and coils.[2] Temperature of the fluid in the belly was allowed to vary in different experiments between 39° and 40°C. without affecting the results. In about one-fourth of the experiments 0.9 per cent NaCl was used as immersion fluid in place of Ringer's, and one experiment was done in which physiological sucrose solution was substituted. In no case were the results altered.

The plunger apparatus was inserted through the anus and the free end of the plunger stitched in place through the wall of the intact colon, by passing the threaded needle through a perforation in the small metal ball which caps the end. Usually a balloon was also inserted into the proximal colon so as to lie from 3 to 5 cm. above the end of the plunger, through an incision near the cecum. In most cases blood pressure was also recorded by carotid cannulation.

In the dogs, ether anesthesia was used except in 6 experiments in which sodium barbital, about 250 mgm. per kgm. body weight, in divided intravenous doses, was substituted. Ether administration was done by the usual method of tracheal cannulation, and connection to an ether bottle. In the cats, barbital was given intraperitoneally, and supplemented by intravenous doses after the belly was opened. All injections in anesthetized animals were made into the exposed external jugular vein.

In the observations which follow we shall refer to movements of the plunger as longitudinal, or distal longitudinal; to pressure changes in the balloons arranged in series on the plunger apparatus as circular or distal circular; and to pressure changes in the balloons lying free in the proximal segment, in which circular and longitudinal components are not separated, as proximal activity (3).

Control activity in anesthetized dogs. Under light or moderate ether anesthesia the colon of the dog shows strong longitudinal activity in the distal segment (fig. 2). Six experiments were done in which the animal was allowed to remain undisturbed except for changes in depth of

[2] The mechanical details of the circulating system were supplied by Mr. Sidney Smith, Jr.

anesthesia until death. These tracings, which lasted from 6 to 8 hours, show longitudinal activity more or less continuously throughout. The balloons, on the other hand, in both the proximal and the distal segment, are usually quite inactive in such tracings. There is none of the marked periodicity in the longitudinal record which is characteristic of the unanesthetized dog, although the character and rate of the individual contractions on the longitudinal tracing are similar to those of the unanesthetized animal.

When the depth of anesthesia was increased, the longitudinal activity was quickly depressed, until the entire color, both balloon records and longitudinal tracing, showed complete quiescence. Decreasing the depth of anesthesia was followed by return of activity in the longitudinal record within 2 to 5 minutes, and often without any marked change in carotid blood pressure (fig. 1). In those instances in which the balloons were also active under light anesthesia, it was observed that in rate of contractions, periodicity, and tone changes, the balloon lying in the proximal colon corresponded with the plunger rather closely, while the balloons lying below the plunger, in the distal colon, were either completely quiet, or showed negative pressure waves coinciding with longitudinal contractions (fig. 2). In some cases, the balloon lying just below the end of the plunger, (highest in the distal group) showed activity corresponding to that of the plunger and to that of the proximal balloons, while those lying lower in the distal group were either quiet or showed negative waves. Under ordinary anesthesia, there was never any activity in the lower distal balloons of sufficient magnitude to be comparable to that in the unanesthetized dog.

In the dogs which were anesthetized with barbital, there was no gross difference in either longitudinal or circular activity in the distal segment, or balloon activity in the proximal segment, from those in which ether was used (fig. 3). In the work which follows, ether was the anesthetic of choice, as we were able, with it, to obtain control activity of almost any desired magnitude by modification of the depth of anesthesia.

Two cats were used, in which the anesthetic was barbital. Control activity was similar to that in the anesthetized dog. It is not possible, at this time, to make a comparison with the unanesthetized cat.

Adrenalin. Ninety-five injections were made in 20 dogs under ether anesthesia; 21 in 6 dogs under barbital; 65 in 27 experiments on 6 unanesthetized dogs; and 7 in 2 cats under barbital. Doses have varied from 1 cc. of a 1:50,000 solution to 1 cc. of a 1:1000 solution. The usual dose has been 1 cc. of a 1:2000 solution. In this study the synthetic preparations Adrin (H. K. Mulford Co.) and Suprarenin (Metz Laboratories) were used exclusively.

In the dog under moderate anesthesia, with the longitudinal tracing capable of showing either augmentation or depression, and the balloons

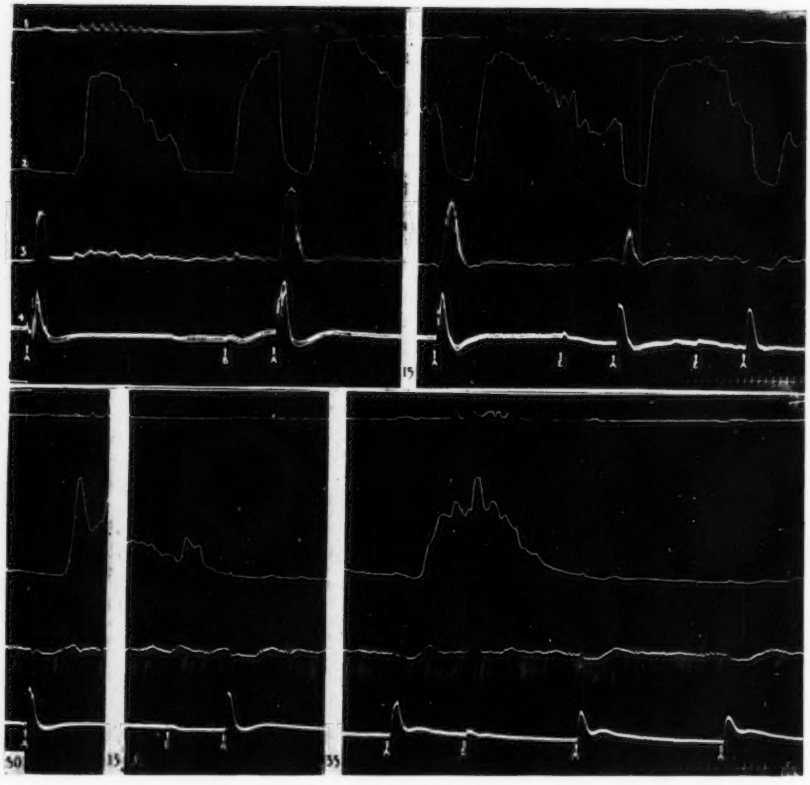

Fig. 1. Dog, 15 kgm. Ether anesthesia. Ringer irrigation at 39°C. *1* is proximal balloon; *2*, longitudinal record of distal colon, plunger fastened in place 15 cm. above internal anal sphincter; *3*, balloon record of distal colon, 6 cm. above internal anal sphincter; *4*, carotid blood pressure. Error in vertical alignment of records less than 5 seconds. Time tracing in minutes. At the points marked *A* 1 cc. adrenalin 1:2,000 was given. At the points marked *E* 1 mgm. ergotamine was given. At *B* the anesthesia was lightened, with almost immediate augmentation of longitudinal tone, following which subsequent adrenalin injections show pronounced preliminary depressant effects on the longitudinal. The first injection of adrenalin was given on prolonged longitudinal depression as a result of deep anesthesia, and shows longitudinal augmentation after a latent period of about 6 minutes. Between the 5th and the 6th adrenalin injections (about 1 hour) the anesthesia was deepened to obtain a depressed longitudinal tone for subsequent injections. After the 2nd ergotamine injection, adrenalin no longer produces augmentation of the distal balloon (no. 3), but still evokes the original longitudinal response. Adrenalin given shortly following the 3rd and 4th ergotamine injections (10 to 15 minutes) fails to elicit longitudinal augmentation, but this response returned when 63 minutes was allowed to elapse between the 3rd ergotamine injection, and the 2nd subsequent adrenalin.

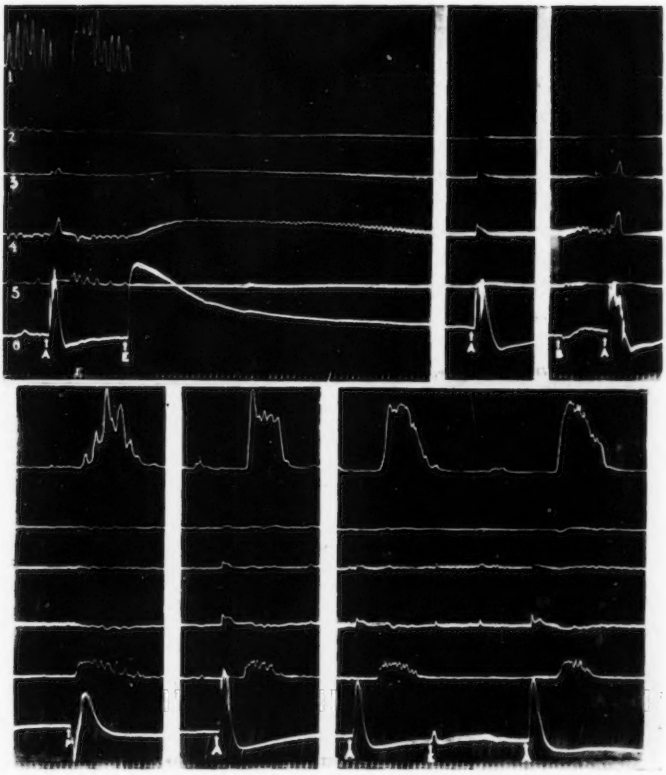

Fig. 2. Dog, 17 kgm. Ether anesthesia. Ringer irrigation at 39°C. *1* is longitudinal record of distal colon, plunger fastened in place 15 cm. above internal anal sphincter. *2*, *3*, and *4* are serial balloons in the distal colon, *4* just above the internal anal sphincter, *2* highest in the distal segment, about 5 cm. below the plunger. *5* is a proximal balloon, and *6* carotid blood pressure. Time tracing in minutes. Error in vertical alignment of records less than 5 seconds. Control activity before the first injection shows continuous longitudinal activity under moderate anesthesia. The sphincter balloon (no. 4) shows negative waves correlated with positive waves on balloon 2. Balloon 3 is almost quiet. At the points marked *A* 1 cc. adrenalin 1:2000 was given, at the points marked *E* 65 mgm. ephedrine, and at the point marked *P*, 5 mgm. pilocarpine. At *B* anesthesia was lightened until the palpebral reflex was easily elicited, with resumption of previous anesthetic level after the next following injection. The first adrenalin injection is followed by the typical diphasic response. In the second phase the longitudinal tone is augmented over its active control. The first ephedrine injection produces augmentation in balloons 3 and 4, with depression in all other records. The next two adrenalin doses show the circular response in balloons 2, 3, 4, and 5, but there is no longitudinal augmentation, even in the extremely light anesthesia following *B*. Pilocarpine produces simultaneous augmentation on all records, most marked on the longitudinal and the proximal balloon. With the next adrenalin injection (4th) 210 minutes after ephedrine, the typical diphasic response is obtained. This is repeated in the 5th. A second ephedrine injection fails to produce maintained elevation of blood pressure, to produce any alteration in motility, or to influence a subsequent adrenalin response.

quiet, the immediate effect of intravenous injection is depression of the longitudinal activity coincident with the blood pressure rise, and coincident with marked augmentation of the balloons (fig. 3). The augmentation of the balloons is most marked in the region of the anal sphincters, but is definite even on the proximal balloons. The effect on the balloons is characteristic, and can be subdivided into two parts (fig. 4), the first of which is a smooth, sudden rise in tone lasting from ½ to 1 minute. The second rise follows after a slight drop in tone, and consists of a high, well-

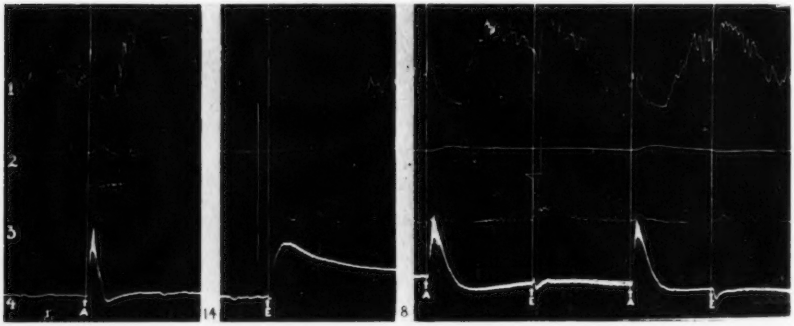

Fig. 3. Dog, 12 kgm. Sodium barbital anesthesia (3 gm.). Ringer irrigation at 39.5°C. *1* is longitudinal record of distal colon, plunger fastened 15 cm. above internal anal sphincter. *2* is balloon in distal segment, 5 cm. above internal anal sphincter. *3* is a proximal balloon, and *4* carotid blood pressure. Error in vertical alignment of records less than 10 seconds. Time tracing in minutes. At the points marked *A* 1 cc. adrenalin 1:2000 was given, at the points marked *E* 65 mgm. ephedrine. The first adrenalin injection gives typical diphasic response. Circular augmentation during the first phase is well marked in the proximal balloon. First ephedrine produces longitudinal depression with circular augmentation, with quick recovery. A second adrenalin injection 27 minutes after ephedrine, when motility has returned to control conditions, gives a typical response. Second and third ephedrine injections produce slight depressions in blood pressure, with slight augmentation of longitudinal tone and motility. Adrenalin shortly following (12 minutes) the second ephedrine injection, produces typical diphasic effect.

marked tone change, usually much higher than the first, upon which are usually superimposed well-formed type I contractions. These two effects occupy from 2 to 4 minutes. When the two parts of the effect are easily separated on the balloon records, it can be seen that the first, smooth tone rise is concurrent with the rise in blood pressure, while the second, characterized by the augmented type I contractions, occurs during the return of blood pressure to normal (fig. 2).

Then follows a period of quiet, lasting from 1 to 5 minutes, on the balloons. During this phase, the longitudinal suddenly becomes active,

and invariably is augmented in tone and activity over its control. The augmented longitudinal activity lasts from 4 to 20 minutes, with return to the control level. During the period of augmented longitudinal activity the proximal balloon is usually also augmented, while the balloons below the plunger are irregular in behavior, sometimes being completely depressed, sometimes being augmented. In the latter event, waves of contraction can frequently be traced over the distal set of balloons in a peristaltic direction.

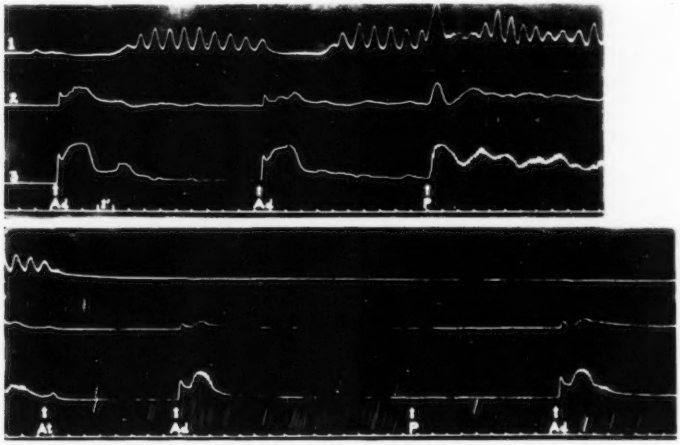

Fig. 4. Dog, 15 kgm. Ether anesthesia. Nine-tenths per cent NaCl immersion fluid. Temperature kept between 38° and 39°C. by large electric lamp. *1* is longitudinal record of distal colon, plunger fastened 15 cm. above internal anal sphincter. *2* and *3* are distal balloons, *3* just above internal sphincter, and *2* 3 cm. above. Error in vertical alignment not greater than 15 seconds. Time tracing in minutes. At the points marked *Ad* 1 cc. adrenalin 1:1000 was given, at *P*, 3 mgm. pilocarpine, and at *At*, 3 mgm. atropine. The first two adrenalin injections evoke typical responses. Pilocarpine given during the 2nd phase of the response to the 2nd adrenalin injection produces immediate augmentation on both longitudinal and balloon records. Following atropine adrenalin produces only the first phase of the typical response, longitudinal augmentation being absent. Pilocarpine following atropine fails to augment either circular or longitudinal.

If the depth of anesthesia is increased, so that there is no longitudinal activity appearing on the record, the delayed augmentory effect is more striking (fig. 1). Following the injection, with a latent period of from 3 to 10 minutes, there is a sudden activation of the longitudinal, with a tone rise and contractions lasting as long as 20 minutes. The circular response, and its relationship to the longitudinal and the blood pressure, is the same as in moderate anesthesia. With very light anesthesia, the imme-

diate depressant effect on the longitudinal is made more striking, without altering in any other particular the total response (fig. 1).

These effects were duplicated on cats under barbital, and on dogs under barbital.

On the unanesthetized dog the response was essentially the same, with the exception of some of the time relations. There was almost no latent period between the circular augmentation and the longitudinal augmen-

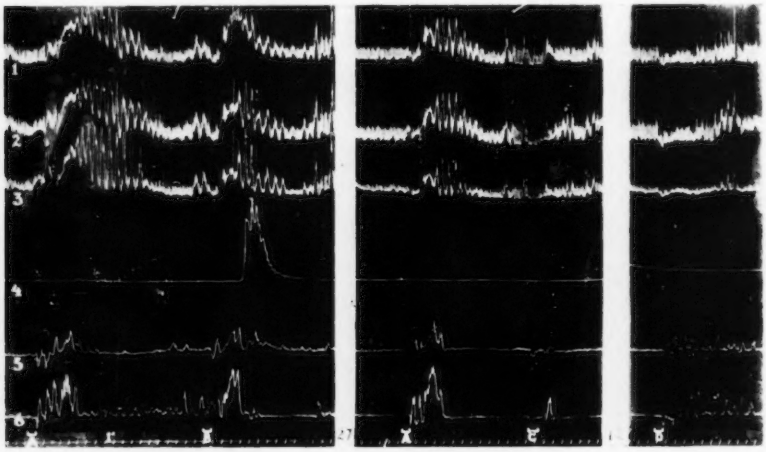

Fig. 5. Dog, 20 kgm. Unanesthetized. Colon transected 15 cm. above left colic vessels, colostomy and distal colon pouch. *1*, *2*, and *3* are balloons in the proximal segment at intervals of 5 cm. *4* is longitudinal record of the distal pouch, and *5* and *6* are balloons in the distal pouch, *6* just above internal sphincter, and *5*, 3 cm. above. Error in vertical alignment less than 5 seconds. Time tracing in minutes. At the points marked *A*, 5 cc. adrenalin 1:50,000 were given, at *B*, 10 cc., and at *C*, 1 cc. of the same. At *D*, 10 cc. 0.9 per cent NaCl were given in the same manner as all other injections. With the 5 cc. dose, *A*, the response is typical except that the longitudinal augmentation is lacking during the second phase. With the 10 cc. dose, the complete response is obtained. The 1 cc. dose has no effect. Injection of saline has an effect on the distal balloons totally unlike the effect of adrenalin. (This effect was not constant, many of the animals giving no motility response to the injection of saline controls.)

tation. The division of the immediate circular augmentation into two parts was more difficult, the whole effect on the balloons in this phase, in both proximal and distal segments, lasting about 3 or 4 minutes (figs. 5, 6). Coincident with the termination of the balloon activity, the longitudinal was augmented in both tone and activity. During the augmented phase of the longitudinal, proximal balloons were also augmented, frequently showing peristalsis, and, in other cases, where peristalsis did not

appear, showing high, well-formed, regularly rhythmic type II contractions. During this period, in which the proximal balloons and the distal longitudinal were active, the distal balloons were quiet. Due to the absence of an interval between the immediate (circular augmentory) and delayed (longitudinal augmentory) effects, the proximal balloons usually show, in the unanesthetized dog, continuous augmentation following the injection. Coincident with the longitudinal augmentation and the distal circular depression, however, there is a marked change in character of the

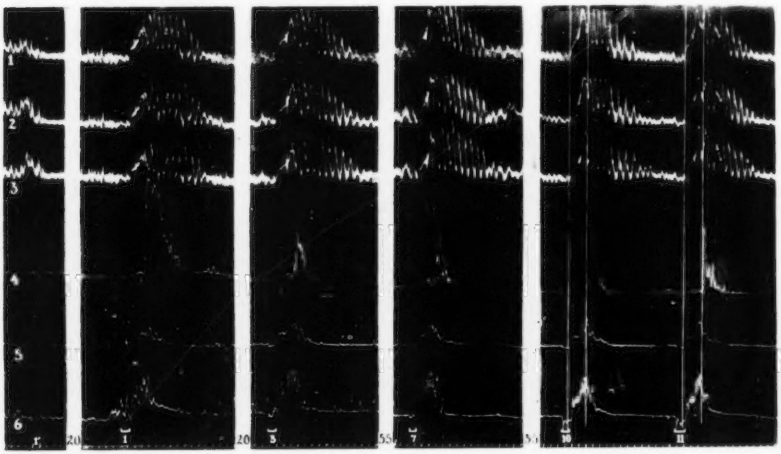

Fig. 6. Preparation and records the same as figure 5. At the points numbered and indicated by bars, 15 cc. adrenalin 1:50,000 were given. These are extreme responses in a series of 11 injections during the course of a single tracing, given at approximately 20 minute intervals. The serial number of the injection is given below the bar marking the injection. The vertical lines drawn through responses to injections 10 and 11 divide the response into the two phases, the first line signalling beginning of the first phase, the second the beginning of the second. The first phase is well marked in the proximal segment. Error in vertical alignment of records not greater than 10 seconds.

proximal activity (fig. 6). During the distal circular phase, the proximal balloons show essentially the same type of activity as the distal circular records, i.e., high type I contractions on an irregular tone. Coincident with the longitudinal augmentation in the distal pouch, and with the circular depression, however, there is an immediate alteration of proximal activity into the smooth, regular type II contractions mentioned above.

Repeated doses of adrenalin in both anesthetized and unanesthetized animals had essentially the same effect as the first injection. On un-

anesthetized dogs as many as 11 doses have been given at intervals of about 20 minutes without variation in the results (fig. 6). If the second dose was given during the period of augmentation of the proximal balloon and the longitudinal from a preceding injection, the effect was the same as if the activity were spontaneous, i.e., there was depression of the longitudinal, with augmentation of the circular, followed, after an interval, by augmentation of the proximal and the longitudinal over the activity preceding the injection (fig. 1, fig. 4).

On one dog, unanesthetized, a series of varying dosages was given, in an attempt to establish roughly threshold concentrations for these effects (fig. 5). It was found that 5 cc. of a 1:50,000 solution produced typical immediate augmentation of the circular, in both distal and proximal segments, followed immediately in the proximal balloons by a period in which there were high, regular type II contractions, frequently peristaltic, but that the augmentory effect on the longitudinal was lacking. Ten cubic centimeters of the same solution produced augmentory effects on both circular and longitudinal as described above. One cubic centimeter of the solution had no definite effect, although there was a slight, atypical augmentation of the circular. The latter was no greater than a similar augmentation of the circular sometimes seen when equal volumes of physiological salt were given.

In unanesthetized dogs, in addition to the typical effects described, with large doses (1 cc. of a 1:1000 solution) sometimes defecation movements were elicited, and sometimes vomiting. In dogs with intact colon (6-balloon technique) actual defecation was sometimes obtained with such doses. The description given above applies only to the effect as seen uncomplicated by these factors. In the cases described as exhibiting the typical uncomplicated effect no skeletal muscle movements could be detected, and no gross symptoms following the injection except for alterations in respiratory rate and heart rate.

Pilocarpine. A total of 35 injections was given in 14 dogs under ether or barbital anesthesia, and 18 in 6 dogs without anesthesia. Doses varied from 1 to 10 mgm. in 0.1 per cent isotonic solution.

In all cases the effect was augmentation of both the longitudinal and circular of the distal segment, and augmentation of the proximal balloons. There was no division of the effect into phases as in the case of adrenalin, the augmentation appearing nearly simultaneously on all the records. Serial balloons in both the proximal and the distal segment showed definite peristalsis in most instances (figs. 2 and 4). Even the first contraction was frequently traceable as a wave of contraction moving toward the anus. Where peristalsis could not be detected, the contractions were high, regularly rhythmic type II contractions, resembling in character the contractions appearing during the period of proximal-longitudinal augmentation

following adrenalin. The effect was usually complete within 15 to 30 minutes.

A second injection given before termination of the effects of the first was followed by still further augmentation without appreciable latent period, and without preliminary depression in any of the records.

Pilocarpine given before the augmentory effect of adrenalin on the proximal and the longitudinal was complete was followed immediately by still further augmentation without preliminary depression or appreciable latent period (fig. 4).

Adrenalin given before the augmentory effect of pilocarpine was complete had the same effect as if given during a period of spontaneous activity, preliminary augmentation of the circular with depression of the longitudinal, followed by augmentation of the longitudinal and the proximal.

Ephedrine. A total of 8 injections was given in 4 dogs under ether or barbital anesthesia. Ephedrine sulfate, 65 mgm. (Eli Lilly & Co.) made up in about 5 cc. of isotonic salt solution was given at each injection.

The first injection was invariably accompanied by depression of the longitudinal and augmentation of the circular lasting approximately as long as the elevation of blood pressure (figs. 2 and 3). The augmented circular activity was most marked in the lower colon, and differed from the circular augmentation after adrenalin in developing more slowly and being maintained much longer. It was not possible to separate this augmentation into two parts, as in the case of adrenalin. The augmentation was characterized by well-formed type I contractions, as in the adrenalin effect. The longest duration of this effect obtained was more than 90 minutes.

A second ephedrine injection given even as late as 50 minutes following the first, failed to produce either a rise in blood pressure, or depression of the longitudinal. In three cases, where second or third injections produced a slight fall in blood pressure, the effect on the longitudinal and the proximal was augmentation rather than depression, and the distal circular augmentation was absent (fig. 3).

When adrenalin was given before recovery of longitudinal-proximal activity after a first injection of ephedrine, the augmentory effect on the longitudinal-proximal was not obtained, but the immediate circular augmentation was still elicited (fig. 2). If, however, adrenalin was administered after recovery of motility from the ephedrine effect, the typical diphasic augmentory effect on both circular and longitudinal-proximal was obtained (fig. 3). A dose of adrenalin given shortly following a second or third, non-depressant ephedrine injection elicited a response complete in all phases (fig. 3). Blood pressure response to adrenalin was not altered by previous ephedrine in either case.

Atropine. Injections of widely varying amounts of atropine sulfate up

to 250 mgm. were made in 10 anesthetized dogs. The solution used was a 0.1 per cent isotonic solution. Atropine was also given in amounts up to 5 mgm. in unanesthetized dogs.

The criteria used for complete atropinization of the peripheral mechanism were failure of response to pilocarpine, and failure of activation with very light anesthesia. This point was reached before death of the animal in only 4 cases. It was noted that when atropine paralysis of the colon was obtained, it was with moderate doses, 2 to 10 mgm. If doses of this size did not produce paralysis, larger doses also failed. In about half the cases, the 2 or 4 mgm. dose given repeatedly to reach the desired cumulative effect was followed by temporary augmentation on all the records, frequently only a single contraction.

When complete atropine paralysis was obtained, according to the above criteria, adrenalin failed to produce any activation of the longitudinal, while the immediate augmentory effect on the distal circular was preserved without apparent diminution (fig. 4). Pilocarpine at this stage failed completely to activate either the longitudinal or circular of the distal colon, or the proximal balloon.

Complete atropine paralysis was not obtained with the doses given in any of the unanesthetized animals. After small doses (2–3 mgm.) there was usually some diminution in both longitudinal-proximal and circular activity, and some diminution in the longitudinal-proximal augmentation following adrenalin, but never the complete suppression of the latter obtained in the acute animals with more complete paralysis.

Ergotamine. Ergotamine tartrate (Gynergen "Sandoz" in 1 cc. ampules containing 0.5 mgm. each) in doses of 1 mgm. was given to 6 dogs under anesthesia. The dose was repeated until the effects given below were obtained, usually after a total of 2 to 4 mgm. had been given at intervals of 30 minutes or less.

The immediate effect of such a dose was not marked. Usually there was a slight diminution in longitudinal-proximal activity, with a possible slight augmentation of the distal circular. In all cases, however, after 2 to 4 mgm. of ergotamine had been given, subsequent adrenalin injections failed completely to elicit the immediate augmentory effect on the distal circular, while the longitudinal depression followed by longitudinal augmentation was preserved (fig. 1). In place of the original immediate augmentation of circular tone and activity during the longitudinal depression, after ergotamine adrenalin produced an immediate and marked depression of tone in the circular record, concurrent with longitudinal depression. The second phase of the adrenalin effect, consisting of augmentation of the longitudinal and proximal, followed without marked alteration in time relationships or intensity. However, for a period as long as 20 minutes following increased doses of ergotamine, adrenalin

failed to augment the longitudinal also. Within 30 to 40 minutes, after ergotamine in such doses, adrenalin had recovered its augmentory effect on the longitudinal and the proximal, while the immediate augmentory effect on the circular was still entirely absent.

It was not possible, with such doses of ergotamine, to detect any alteration in the blood pressure response to adrenalin.

Discussion. The differences in activity of the colon under anesthesia are in line with the observations of Miller (5) except for the important particular of longitudinal activity. Whereas the unanesthetized animal shows periodic longitudinal activity, with long intervening periods of quiet, such periodicity is almost lacking in the anesthetized animal. Under moderate anesthesia, total longitudinal activity is much greater than in the unanesthetized dog. Miller reported a stimulating after-effect of ether anesthesia. This might be interpreted as indicating stimulation masked by depression. It is possible that such an effect is sufficient to stimulate the longitudinal layer to overactivity, under light or moderate anesthesia. The possibility that the stimulation arises from our surgical procedures and the exposure of the viscera, with retention of considerable irritability in the longitudinal layer under moderate anesthesia, cannot be ruled out at present, although care was taken to protect the gut from such stimuli. Whatever the origin of the continuous longitudinal activity, it is significant that associated with it is almost complete quiet in the circular layer. Whether this indicates release of the longitudinal from the inhibiting influence of the circular mechanism, or depression of the circular from overactivity of the longitudinal mechanism, or even that the conditions cited above have opposite effects on the two layers, it is not possible to say.

That adrenalin under some conditions may exert a motor influence on the gut has been sporadically reported (6), (7), (8). Recent work indicates that previous parasympathetic stimulation is necessary in order that such an effect be obtained (9), (10), (11). However, in the colon of both the cat and the dog, under our conditions, such stimulation was not necessary. It seems to be necessary only that the longitudinal layer be capable of activity. Paralysis due to deep anesthesia, atropine, ephedrine, or large doses of ergot, rendered the longitudinal layer incapable of being augmented with adrenalin. It is not necessary, however, for the longitudinal layer to be exhibiting activity at the time, as is shown by the activation with adrenalin from complete quiet in moderately deep anesthesia.

That more than one mechanism is involved in the total response to adrenalin is suggested by the polyphasic character of the response. That the augmentory effect on the circular during the first phase is not merely contraction of the internal anal sphincter as is stated by Learmonth and

Markowitz (12) is shown by the fact that even balloons in the proximal colon show the same effect. This is true also in the colostomized dogs used in the chronic study, where the possibility of a passive effect from the sphincter is ruled out by complete separation of proximal and distal segments. In character, especially in the appearance of the well-defined type I contractions on the tone change, this first effect resembles closely the rhythmic pulsations which we described in the unanesthetized dog. It is clear that the longitudinal layer does not participate in this first effect, which is an additional point of similarity to the rhythmic pulsations. It is during this phase that the longitudinal is sharply depressed, exhibiting a reciprocity with the circular quite like the reciprocity between the longitudinal and the rhythmic pulsations of the circular in the spontaneous motility of the unanesthetized dog.

The longitudinal augmentation, associated with activity in the proximal segment, which follows the first phase, is the most striking part of the effect. Blood pressure by this time is back to normal. The entire picture in the second phase closely resembles spontaneous motility, greatly augmented, and is almost duplicated by activity following pilocarpine. Pilocarpine, injected during this phase, immediately augments still further, without grossly altering the character of the activity. Adrenalin, however, injected during this phase, first depresses the longitudinal and proximal, with augmentation of the distal circular, then augments the longitudinal and proximal above that following the first injection. By administering adrenalin at intervals before the second effect has developed, we have been able to hold out the second effect for as long as 30 minutes. It is clear that the second effect of adrenalin is antagonized by the first effect, and, further, that pilocarpine is not antagonistic, but augmentory, to the second effect.

Atropine, when we were successful in obtaining complete atropinization, further separates the first and second phases of the adrenalin response by allowing the first to develop fully while completely suppressing the second. After atropinization pilocarpine was unable to stimulate any part of the colon, while adrenalin still retained its immediate augmentory effect on the distal circular. From work on the cat's uterus, Cushny (24) states that "Pilocarpine differs from adrenalin in being antagonized completely by atropine, whether it contracts or inhibits the uterus, while the effects of adrenalin or of hypogastric stimulation are not changed in any way by atropine." In the colon, it would appear from our work that the latter part of this statement is true only of the first phase of the adrenalin effect. The second phase is as completely lost after atropinization as is the response to pilocarpine.

There is a complete dissimilarity between the latent effects of adrenalin and ephedrine, although the first effects seem to differ primarily only in

time relations. Following ephedrine, in the time allowed in our tracings, there is no augmented return of the longitudinal and proximal, but a slow recovery of the control activity. This difference between the two drugs has apparently not been observed by other workers, although Kreitmair (13) mentions that both adrenalin and ephedrine produce high tone and contractions in the excised uterus, and Kinnaman and Plant (14) state that ephedrine sometimes increased the tone and contractions in Thiry-Vella loops of the ileum. The latter call attention to "the marked similarity to the effects of epinephrine, in that the activity is decreased." The depressant effect of ephedrine on the longitudinal and the proximal seems proportional to the intensity and duration of the blood pressure effect. Where long maintained high blood pressure was obtained, depression of the longitudinal and the proximal segment was concurrent. On second or third injections, where no blood pressure rise was obtained, there was no depression of motility.

In appearance and relationship to blood pressure, it is apparent from his tracings that the first phase of the adrenalin response was observed by Bunch in 1898 (6) on the small intestine. His tracings, however, fail to show our second phase. His methods were similar to ours in that he examined the gut in situ, and dissimilar in that he did not attempt to separate circular and longitudinal activity. Our separation of distal colon activity into longitudinal and circular components shows clearly that in this region the depressant portion of the response to adrenalin, which is the only response usually observed with other methods, is restricted to the longitudinal layer, and to the type of circular activity associated with longitudinal activity. Circular activity of the sphincter type is augmented.

The second effect is clearly not secondary to the motor response of the circular during the first effect, for after ergot the motor response of the circular is reversed without impairing the delayed effect. The augmentation of the longitudinal, and the activity shown by the balloons during the second phase, are similar in all respects to spontaneous motility, and to motility produced by pilocarpine. The summating effect of pilocarpine during this phase, and the immediate antagonistic effect of adrenalin seem to warrant the conclusion that antagonistic mechanisms are set in activity during the first and the second phase of the response to adrenalin. That the mechanism active during the second phase is the same as that activated by pilocarpine and paralyzed by atropine seems also a justifiable conclusion.

The activity set up throughout the colon as the first effect of adrenalin is so similar in its type and in its reciprocity with the longitudinal to the rhythmic pulsations occurring during spontaneous motility in the unanesthetized dog, as to suggest a similarity in mechanism.

Assuming, with some workers (11), a specificity for adrenalin which may not exist, we should be forced to the following conclusions: 1. The first effect of adrenalin on the colon is augmentation of rhythmic pulsations throughout the colon, a purely circular, sphincter type of activity, apparently under the motor control of the sympathetics. 2. The delayed effect of adrenalin is augmentation of a reciprocal type of activity, peristalsis associated with longitudinal contractions, apparently under the motor control of the parasympathetics. Why uncomplicated stimulation of the sympathetics should be followed by activation of the parasympathetics is a question raised, but not answered, by these conjectures. Weitz and Vollers, in 1926 (8), suggested that "probably in the temporary interruption of peristaltic movement, and the diminution of tone, lies a sufficient basis for the following augmented activity." That their results

TABLE I

Response of the colon to adrenalin

CONDITION	FIRST PHASE			SECOND PHASE		
	Proximal balloons	Distal longitudinal	Distal circular	Proximal balloons	Distal longitudinal	Distal circular
Moderate anesthesia	+ tone type I	−	+ tone type I	+ type II	+	− or type II
Deep anesthesia	+ tone type I	0	+ tone type I	0	0	0
Unanesthetized	+ tone type I	−	+ tone type I	+ type II	+	− or type II
After adrenalin	+ tone type I	−	+ tone type I	+ type II	+	− or type II
After atropine	+ tone type I	0	+ tone type I	0	0	0
After ergot	− or 0	− or 0	− or 0	+ type II	+	− or type II
After ephedrine	+ tone type I	0	+ tone type I	0	0	0

were specific adrenalin effects might be questioned on the basis of inadequate controls.

Data are accumulating which have been interpreted as showing that adrenalin stimulates not only the thoracico-lumbar but also the craniosacral apparatus. Heinekamp in 1925 (15) found that following physostigmine adrenalin produced slowing of the heart in vagotomized animals, which was antagonized by atropine. He concluded that adrenalin acts on both systems, the effect obtained being determined by relative thresholds. Smirnow and Schiroky, in 1926 (16) found exaggerated vagal beats when adrenalin was given following morphine. They concluded from this evidence that adrenalin is an amphotropic hormone, acting on whichever system is the more irritable, morphine serving to increase "vagal tone."

The opposite interpretation, in which the specificity of function of the two divisions of the autonomic system is questioned, might be considered as explaining results obtained from physical stimulation of nerves (17), (18), (19), (20), (21). It would seem, however, an economy of ideas

to withhold such interpretation until the central connections of such peripheral aggregations of autonomic fibres as the vagus and hypogastric are better known.

Following the statement by Dale in 1906 (25) of the specificity of ergot alkaloids for the motor elements associated with thoracico-lumbar innervation, most workers with this drug and its reversal of subsequent adrenalin responses have assumed that the response remaining indicated sympathetic rather than parasympathetic activity (22), (23).

For a clear-cut interpretation of our results in the light of previous work, one must assume either that adrenalin stimulates both sympathetics and parasympathetics; or, that neither the sympathetic nor the parasympathetic is, strictly speaking, motor to the colon, but that each is capable of giving rise to a particular type of motor activity, the two types being mutually antagonistic. It would seem necessary to add, to the latter alternative, that not only are the two types of activity mutually antagonistic in that they are incapable of co-existing in the same segment, but that the suppression of parasympathetic activity during that of the sympathetic is followed by a complete reversal.

Neither interpretation seems complete, nor totally satisfactory.

SUMMARY

1. Adrenalin produces in both anesthetized dogs and cats, and in unanesthetized dogs, a diphasic augmentation of motility in the colon. During the first phase, there is augmentation of stationary circular contractions, a sphincter type of activity, throughout, with depression of longitudinal and the peristaltic type of activity. During the second phase there is complete reversal of this condition, with augmentation of the longitudinal and the peristaltic type of activity, and depression of the sphincter type.

2. Threshold doses of adrenalin produce only the first phase.

3. Atropinization antagonizes the second phase, without diminution or alteration of the first.

4. Ergotamine reverses the first phase, without alteration of the second.

5. Pilocarpine augments the second phase, without preliminary depression, and without producing any change in character of activity.

6. A second dose of adrenalin given during the second phase produces reversal of activity back to that of the first phase, followed by augmented recovery of the second phase.

7. These effects were not obtained with ephedrine, except that the total response is similar in appearance to that of the first phase of the adrenalin response.

The authors wish to express their appreciation to Prof. A. J. Carlson for advice and criticism.

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AN ATTEMPT TO PRODUCE SPINAL CORD DEGENERATION IN DOGS FED A HIGH CEREAL DIET DEFICIENT IN VITAMIN A. THE INCIDENTAL DEVELOPMENT OF A SYNDROME OF ANEMIA, SKIN LESIONS, ANOREXIA AND CHANGES IN THE CONCENTRATION OF BLOOD LIPOIDS[1]

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Received for publication April 18, 1932

The beneficial effect of large amounts of whole liver upon the neurological phenomena of subacute combined degeneration of the cord was demonstrated by Minot and Murphy (1927) and has subsequently been confirmed by others. Ungley and Suzman (1929) have suggested that there may be a factor in liver, distinct from the principle effective in pernicious anemia, which influences the nervous system beneficially and that only a part of the neurological improvement is dependent upon alleviation of anemia and gain in strength. In order to determine whether such a separate factor exists, an attempt has been made to produce in dogs a condition simulating subacute combined degeneration in man.

It was thought that this problem could be approached from a nutritional standpoint, because neurological lesions similar to those of pernicious anemia occur in pellagra, lupinosis, ergotism and the "central neuritis of Jamaica," conditions usually regarded as being dependent on dietetic anomalies. Furthermore, Castle and co-workers (1930) have demonstrated that Addisonian pernicious anemia can be regarded as a dietary deficiency disease conditioned by the state of the gastro-intestinal tract.

Although the nervous lesions which develop with a deficiency of vitamin B are usually confined to the peripheral nerves, lesions resembling those found in subacute combined degeneration may occur in the spinal cord (Vedder and Clark, 1913; Gildea et al., 1930). Furthermore, Eijkman

[1] The expenses of this investigation have been defrayed by the Rockefeller Foundation. We offer thanks to Charles E. Walker, A. Bloomberg, and B. Miller for their assistance in the care of the animals. We are especially grateful to Dr. George R. Minot and Dr. James H. Means for their kindly criticism.

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(1897), Funk (1914), McCarrison (1928) and Vogt-Moller (1931) have shown that the central nervous system symptoms are attributable, not to organic change, but rather to an intoxication of the system from an interference with metabolism or from the ingestion of a toxic factor. Diets deficient in vitamin B were therefore considered unsuitable for the purposes of this experiment.

However, since neurological manifestations indicative of spinal cord involvement, supported in some by histological findings, have been produced in animals on vitamin A deficient diets (Steenbock et al., 1921) usually containing a high proportion of cereal (Hart et al., 1916; Mellanby, 1926, and Hughes et al., 1929); an attempt was made to produce spinal cord degeneration in dogs by means of a similar diet. The inclusion of cereal has been regarded as supplying a positive toxic agent or "toxamin" (Mellanby 1930) inimical to the central nervous system. Although the attempt proved unsuccessful, there developed certain incidental and unexpected manifestations, a description and discussion of which form the main subject of this paper.

EXPERIMENTAL METHODS. Eight adult dogs were fed a basal diet consisting of rolled oats 76.8 per cent, sugar 12.8 per cent, lard 6.4 per cent, bone ash 2.4 per cent, salt mixture[4] 1.6 per cent (Cowgill, 1921) and 10 drops three times weekly of Viosterol in Oil, 250 D, N.N.R. This diet contains protein 12.3 per cent, carbohydrate 64.6 per cent, fat 11.9 per cent and mineral content 5.5 per cent. It was considered that this diet contained sufficient of all known vitamins excepting vitamins A and C. Reasons for the omission of the latter will be given later, and the possible shortcomings of the vitamin content in regard to the vitamin B complex will be discussed forthwith. Two of the animals received in addition a source of vitamin A, in the form of mammalian liver oil or of cod liver oil concentrate. Inasmuch as the object was to produce a toxic effect by excess of cereal, in addition to the effect of avitaminosis A, the dogs were given as much of the above mixture as they would eat. In an attempt to increase the toxic effect still further, an extract of oatmeal was injected into one of the dogs.[5]

[4] The salt mixture consists of sodium chloride 10 grams, calcium lactate 4 grams, magnesium citrate 4 grams, ferric citrate 1 gram, iodine-potassium iodide solution 10 minims.

[5] This was done in view of the work of Teruchi and co-workers (1929). These investigators, having first demonstrated a quantitative relationship between the amounts of polished rice necessary to produce polyneuritis and those of anti-neuritic vitamin sufficient to prevent it, and having subsequently prepared from polished rice an alcoholic extract, which on injection into animals deprived of vitamin B, would produce polyneuritis, concluded that polished rice contains a neuro-toxic factor, the action of which is neutralized by the administration of vitamin B. Thus, applying this rationale to our experiments, large amounts of finely ground whole oatmeal were extracted several times with absolute alcohol, which was then evaporated down in vacuo. The residue, consisting of a dark brown oil, was used for injection into one of the animals.

The animals were kept in individual cages, fed once daily, exercised periodically and weighed at intervals.

In view of the fact that subacute combined degeneration of the cord is almost invariably associated with pernicious anemia, the red blood cell count and hemoglobin concentration were observed with regularity. Further, in view of the paucity of blood lipoid studies during vitamin A deficiency, opportunity was taken to note any possible fluctuations in the blood of the concentration of cholesterol and lecithin. Determinations of the red and white blood cell, hemoglobin, cholesterol and lecithin were made once a week on oxalated venous blood obtained from the femoral vein under uniform conditions, at the same time in the morning in the post-absorptive stage. The cholesterol was determined by Bloor's saponification method (1922), and the lecithin phosphorus according to the method of Whitehorn (1924), both methods found to give consistent values in duplicate when carried out under standard conditions.

Since it is known that complete vitamin deprivation of the body tissues is brought about less readily and with less regularity under given conditions in adult than in young animals, owing to variations in stores and requirements, it was thought desirable to ascertain whether or not vitamin A exhaustion had occurred, by estimating the concentration of this vitamin in the organs after death. For this purpose portions of liver, brain, kidney and spleen removed at autopsy were placed in 50 cc. of a 40 per cent aqueous solution of potassium hydroxide. After saponification, the material was extracted three times with petroleum ether. After washing with water several times until there was no tendency to form an emulsion, and after dehydrating by means of anhydrous sodium sulphate, the petroleum ether layer was evaporated to dryness under partial pressure in an atmosphere of carbon-dioxide. This residue, consisting of the unsaponifiable fraction, was dissolved in a known amount of chloroform and assayed for its vitamin A content by the antimony trichloride method of Carr and Price (1926), using the Rosenheim-Schuster modification of the Lovibund tintometer, the results being expressed in blue units per gram of tissue "per cm. cube," according to the method of Moore (1929).

ANALYSIS OF FINDINGS. Of the eight dogs used, three died before the completion of the experiment, in one case due to distemper, in another due to the effects of the injections of oatmeal-extract, and in the third following the removal of a small portion of the liver for assay of its vitamin A content. In the five remaining dogs, there were no significant differences in the symptomatic manifestations or blood findings between the three dogs fed only the basal diet, and those which received vitamin A in addition. The findings in these dogs, therefore, may be grouped together.

These five dogs lived for periods of from twenty-three to thirty-five weeks on the experimental diet. They ate well for periods varying from eleven

to nineteen weeks and continued to gain weight for periods of from five to eleven weeks. In three, the weight commenced to decline coincident with the onset of anorexia, whereas in two the loss of weight preceded the loss of appetite by some weeks, attributed to attacks of diarrhea. It may be mentioned here that one dog, although dying prematurely as the result of an operation nineteen weeks after the beginning of the experiment, had already shown anorexia and loss of weight commencing as early as the fifth week.

All the five dogs developed dermatitis with loss of hair and in four of these skin ulcers were also present. These skin lesions appeared earlier and were more severe in the two dogs receiving vitamin A. Three types of lesions were present. First, a dry scaly condition with loss of hair; secondly, the occurrence of slightly raised reddened areas on the bony prominences of the limbs and elsewhere; and thirdly, the subsequent development in these latter situations of round or oval ulcers. These were somewhat punched-out and had a flat, fairly clean base from which there was a small amount of brownish discharge. At first about one-half inch in diameter, they had increased at the time of death to from one to two inches across and were deep enough to expose the underlying hard structures. In the later stages the edges of the ulcers became somewhat raised and rounded. It is noteworthy that these lesions showed little gross evidence of inflammatory reaction, there being no signs of local congestion, tenderness, edema or purulent secretion. Repeated treatment with antiseptic lotions and with a proprietary mange cure, although causing some temporary improvement in the scaly skin eruption and allaying irritation, failed to affect the progress of the ulcers.

A purulent discharge was noted in the eyes of the two dogs receiving vitamin A and in one being fed the basal diet alone, but in none was there evidence of xerophthalmia. Apart from some unsteadiness in the latter stages, probably due to extreme weakness, there were no neurological manifestations.

The two female dogs, neither of which received vitamin A, developed a condition of continuous heat, as shown by their attitude towards male dogs, and which persisted to within two weeks of death.

Anemia developed in all the five dogs in which the experiment was completed, the hemoglobin concentration falling to a slightly greater extent than the red blood cell count. This was noticeable by the twelfth, eleventh and twentieth week respectively in the three dogs not receiving vitamin A, whereas in those receiving this vitamin, the onset of the anemia occurred during the twentieth week. In one dog in which the experiment was not completed, some degree of anemia was noticeable by the fifteenth week.

A terminal rise of the cholesterol content of the blood occurred in three dogs, and a fall in one, which change in each case was coincident with the

development of the anemia. In the remaining dog the cholesterol values showed no significant alteration. The concentration of lecithin underwent similar fluctuations in all except one dog in which no significant change was noted. The course of two of these animals is illustrated in the charts.

A careful microscopical study of the nervous system failed to reveal any significant pathological changes. The myelin sheaths were stained by means of the Spielmeyer, Ioyez, Pal-Weigert and Scharlach-R methods,

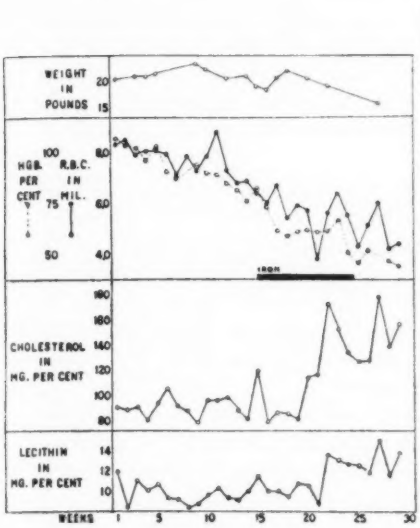

Chart 1

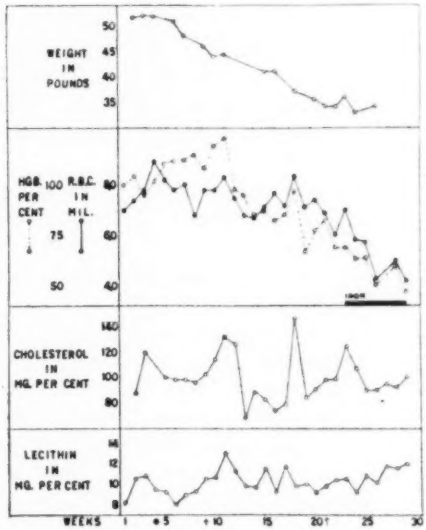

Chart 2

Chart 1. Body weight, hemoglobin concentration, red blood cell count and lipoid content of blood in a dog on basal diet without vitamin A. Skin rash and ulcers appeared during the last two weeks of life.

Chart 2. Body weight, hemoglobin concentration, red blood cell count and lipoid content of blood in a dog receiving the basal diet with vitamin A.

* First appearance of skin rash.

\+ First appearance of purulent conjunctivitis.

↑ First appearance of skin ulcers.

the axis cylinders by the technique of Davenport (1929), the nerve cells by means of cresyl violet, and the hemotoxylin-and-eosin method was used as a general stain. The only positive histo-pathological finding in the nervous system was the presence, in the cerebral cortex, of degenerative swelling of the oligodendroglia, which condition is considered by Wolff, Reed and Cobb (1928) to be indicative of a severe intoxication.

Apart from the presence of terminal broncho-pneumonia and of hyper-

plasia of the bone-marrow, no significant gross or microscopical changes were evident. The body fat, when present, was snow-white in colour.

The tissues of the animals receiving the basal diet alone, and in which the experiment was completed, contained no vitamin A, whereas those receiving this vitamin showed an abundance in the liver and small amounts in the kidney and spleen.

Discussion. Two chief points of interest arise. First, the fact that no neurological changes occurred, and secondly, the occurrence of anorexia, emaciation, skin lesions, anemia and alterations in the concentration of the blood lipoids. Since these manifestations occurred whether or not vitamin A was given, it is obvious that some other deficiency must have been present in the basal diet. Therefore, before the significance of these results can be discussed, it is necessary critically to consider the composition of the diet.

It was estimated that the *caloric value* of the diet was approximately 120 calories per ounce of mixture, and that during the periods of good appetite the daily consumption was half an ounce per pound body weight. The daily caloric intake (approximately 60 calories per pound body weight), therefore, was far in excess of the basal requirements (approximately 35 calories per pound body weight). However, during the latter part of the experiments, when the appetite had diminished considerably, the caloric intake was less than the basal requirements.

The *protein* content of the diet was 12.4 per cent which, for biologically adequate protein, is generally accepted as being sufficient for the maintenance of health in adult animals. That the oat kernel can furnish all the essential nitrogenous units provided its concentration of protein is adequate, namely, 10 per cent, has been pointed out by Osborne and Mendel (1920). In the nutrition of the adult human, the adequacy of the oat protein has been demonstrated by Sherman and co-workers (1919). Moreover, Luers and Siegert (1924) have shown that the protein of oatmeal contains very liberal amounts of the essential amino-acids, and Smith and Hendrick (1926) have demonstrated that, when properly supplemented with accessory food factors, it is just as satisfactory as casein in the nutrition of the rat.

The *salt mixture* has already been shown by Cowgill (1921) and by other investigators (Gildea et al., 1930) to be adequate for the maintenance of health in dogs. The iron content of the basal diet, excluding the small amounts present in oatmeal (3.8 mgm. per 100 gm.), was estimated at 0.0184 per cent. That is to say, the amount of metallic iron ingested during the periods of normal appetite was equivalent to 2.7 mgm. per pound body weight daily, or an average of 98 mgm. per dog daily. In view of the development of anemia, and to obviate the possibility of an iron deficiency dependent on the diminished food intake in the latter parts of the experiments, additional amounts of iron were administered in the form of iron

and ammonium citrate (10 gr. daily, equivalent to 108 mgm. of metallic iron). That these amounts were sufficient is borne out by the work of Whipple and Robscheit-Robbins (1930), who have shown that for maintenance of the hemoglobin level dogs require 20 mgm. of metallic iron daily, and further, that the optimum necessary for regeneration of hemoglobin in anemia due to blood loss is an additional 40 mgm. of metallic iron daily. Moreover, according to Riecker (1930), the optimum daily amount of metallic iron required by the dog for regeneration of hemoglobin after bleeding is 57.5 mgm.

With regard to the presence in oatmeal of the *vitmain B complex*, it is well known that abundant anti-neuritic vitamin B_1 is present. Although Smith and Hendrick (1926) have shown that oatmeal is relatively deficient in the growth-promoting heat-stable factor of vitamin B complex, we originally believed that the large proportion of oatmeal in the diet would supply sufficient of this factor for maintenance of health in adult animals, and that it would therefore be unnecessary to supplement the basal diet with some other source of this factor, such as yeast. Moreover, there is evidence that less of the heat-stable component is required when the protein content of the diet is relatively low (Hartwell, 1924), as was the case in our basal diet. In the light of subsequent events in our experiments, however, it seems probable that there was present in the basal diet given our dogs a relative deficiency of some portion of the vitamin B complex other than the anti-neuritic. Since none of the dogs developed any lesions in the mouth, it is unlikely that the diet was deficient in the so-called "anti-black-tongue" factor, described by Goldberger and associates (1928). Nevertheless, there may have been lacking still other components of the vitamin B complex, the composite nature of which has been demonstrated by several investigators (Hunt 1928, 1931; Reader, 1929, 1930; Williams and Waterman, 1928; Chick and Copping, 1930; Coward et al., 1929).

Most sources of vitamin C, such as orange and lemon juice, contain significant amounts of vitamin A (Willimott, 1928). For this reason, and because there is experimental evidence to show that dogs on a diet lacking in vitamin C do not develop scurvy for periods of from seven to ten months (Lavialle, 1927), it was decided to omit this factor from the basal diet.

As already stated, *vitamin D* was administered in the form of irradiated ergosterol (Viosterol). Oatmeal, as in the case of other cereals, contains abundant *vitamin E*.

Absence of neurological changes. Since several observers, using a vitamin A deficient diet usually containing a high proportion of cereal, have noted neurological symptoms in animals and in some have demonstrated pathological changes in the central nervous system, the question arises as to why no such changes occurred in our dogs. There are several possible explanations.

In the majority of previous experiments young animals were used. We desired to make a comparison with human subacute combined degeneration of the cord, which occurs only in adults, so that grown dogs were purposely chosen. The explanation might lie in the fact that adult animals are less susceptible to vitamin A deficiency than are young growing animals, the stores of which are more rapidly depleted, and, therefore, spinal cord degeneration may require a much longer time to develop than is the case in young animals.

It is not improbable that spinal cord degeneration does not develop until complete exhaustion of vitamin A has been present for a considerable period of time. Since death occurred from causes not attributable entirely to vitamin A deficiency, it is almost certain that the animals would have lived longer had this vitamin been the only limiting factor in the basal diet. That the vitamin A in these dogs had become exhausted only a short time before death is suggested by the observation that in one of the animals which died after nineteen weeks, the liver still contained appreciable amounts of this vitamin.

It is possible, moreover, that in the presence of other deficiencies the specific effects of avitaminosis-A may fail to become manifest. In addition, it is likely, in view of the suggestion of Underhill and Mendel (1928) and of Wolbach and Howe in connection with vitamin C (1926), that the breaking down of tissues which accompanied the emaciation may have liberated some vitamin A. Further, the small amounts of oatmeal ingested in the latter half of each experiment as a result of the anorexia, may have been insufficient for the development of cord degeneration, since, according to the observations of Mellanby (1926, 1930), the production of such lesions seems to depend not only upon the lack of vitamin A, but also upon the toxic effect of large amounts of cereal in the diet.

Consideration of positive findings. The significant changes which occurred during the administration of the diet, were: anorexia, loss of weight, skin lesions, eye infections, prolonged heat, anemia and changes in the lipoid content of the blood.

Since these manifestations occurred irrespective of whether or not the dogs were receiving vitamin A, it is clear that some other deficiency or adverse factor must have been present in the basal diet.

Although *anorexia* developed gradually after periods varying from ten to nineteen weeks, the dogs ate heartily before this time and gained weight for varying periods, the diet apparently being palatable and of sufficient caloric value. It has been shown that the striking anorexia, which follows deprivation of the vitamin B complex in dogs and other animals, is a characteristic of lack of the antineuritic component (Bing and Mendel, 1929; Cowgill et al., 1931), but the diet in the present experiments contained an abundance of this latter constituent. The loss of appetite which develops

in the dogs deprived of the heat-stable, "black-tongue-preventing" factor of the vitamin B complex has been shown by Goldberger and associates (1928) to be attributable to the occurrence of severe mouth lesions, which, as has already been stated, were totally absent in our dogs. Nevertheless, the anorexia in our dogs might have been due to the relative deficiency in oatmeal of the heat-stable growth-promoting factor of Smith and Hendrick (1928), the lack of which has been shown to cause loss of appetite in rats.

The *emaciation* was obviously due, in part at least, to the small food intake dependent on the anorexia, which latter, as suggested above, was perhaps the result of some deficiency in the diet. However, it has been shown that not all the loss of weight which occurs during vitamin deprivation is due to anorexia and low food intake. For example, in deprivation of the vitamin B complex, Stucky and Rose (1929) have shown that loss of weight of dogs deprived of this vitamin was greater than that of control animals ingesting exactly the same amounts of food and water.

With regard to the occurrence of *skin lesions* in animals on deficient diets, it may be noted that in the experiments of Goldberger and associates (1928), dogs, which developed so-called "black-tongue" on a diet deficient in the heat-stable component of the vitamin B complex, showed no dermatitis except in the scrotum. However, on a similar diet, Gildea, Kattwinkel and Castle (1930) reported the occurrence of a generalized mange-like scaly skin eruption with loss of hair. Underhill and Mendel (1928) while experimenting with the Chittenden-Underhill diet noted skin rash and loss of hair in dogs, the occurrence of which seemed independent of the development of the typical mouth lesions, and which could be prevented and sometimes alleviated by the administration of yeast. It is well known that rats fed a diet deficient in the heat-stable components of the vitamin B complex, not only fail to grow, but usually develop a typical dermatitis with loss of hair (Lavialle, 1927; Chick and Roscoe, 1927). The work of Cowgill, Stucky and Rose (1929) is of especial interest in that, during the course of the administration of a diet deficient in the vitamin B complex, dogs developed characteristic symmetrical ulcers on bony prominences which, from their description, are very similar to those noted in our dogs. These various observations suggest that the skin lesions in our dogs may have been due, in part at least, to a deficiency of some component of the vitamin B complex other than vitamin B_1.

The *eye infections*, consisting of a simple purulent conjunctivitis, obviously cannot be attributed to vitamin A deficiency, since two of the animals were receiving this vitamin in their diet and at death had an abundance in the liver. Although the cause of these eye lesions is not clear, it is probable that they were the result of direct extension from the several septic skin lesions which were particularly marked in these dogs. In view of the possible deficiency of some portion of the vitamin B complex in the basal diet,

it is of interest that Goldberger and Wheeler (1928) noted ophthalmia in a proportion of dogs fed a diet deficient in the heat-stable factor of the vitamin B complex. It should be recalled that in our experiments xerophthalmia did not occur in the animals deprived of vitamin A. However, as has been shown in rats by Osborne and Mendel (1921), in guinea pigs by Wolbach and Howe (1928) and in monkeys by Tilden and Miller (1930), the development of this condition during vitamin A deprivation is not a constant feature. This is especially the case in mature animals, either because they have greater stores of the vitamin, or because their requirements are less (Sherman and Smith, 1931).

Concerning the occurrence of *continuous heat* in the two female dogs deprived of vitamin A, it is difficult to understand why this should have happened. Heat in the dog, a monoestrous animal, occurs for a short period, from seven to ten days (Marshall and Jolly, 1905; Evans and Cole, 1927), in each breeding season, of which there are two annually, one each in the spring and autumn. In these two animals, however, active heat, as judged by their attitude toward male dogs was present from August to February, a period of six months. There is no evidence that vitamin A deficiency causes prolonged oestrus in other animals as rats (Evans, 1928) the cornified vaginal cells usually present being most probably an expression of the general cornification of epithelium which occurs in the course of this deficiency (Wolbach and Howe, 1925). We are unable, therefore, to explain the occurrence of protracted heat in our dogs on the basis of a lack of vitamin A. It also cannot be said whether or not the other shortcomings of the basal diet were involved in the production of this unusual phenomenon.

The development of a severe *anemia* in all the dogs in which the experiments were completed is considered the most important positive finding. As has already been stated, the diet contained sufficient minerals to maintain health in dogs, and moreover, the addition of an extra amount of iron did not prevent the progress of anemia, so that a deficiency in the diet of this inorganic constituent cannot be held responsible. However, in view of the work of Hart and co-workers (1925) who have shown that the utilization of iron is dependent on the presence in the diet of certain organic substances, such as cabbage, alcoholic extract of cabbage or chlorophyll, it is possible that the lack of some such substance from our diet may have played a rôle in the development of anemia by rendering the ingested iron unavailable for hematopoiesis. If this substance is a pigment, as has been inferred by those investigators, then the possibility of it having been absent from our diets is suggested by the fact that the small amounts of fat present in our dogs at autopsy were devoid of pigment, being snow-white in colour. Furthermore, Mouriquand and associates (1925) have made the important observation that the iron content of the blood in guinea pigs

with scurvy is considerably lower (0.21 to 0.4 gm. per kgm. body weight) than that of normal animals (0.53 gm. per kgm. body weight), and that the low concentration of iron in the blood returns to normal shortly after the resumption of an anti-scorbutic diet. It is therefore possible that, although adequate amounts of iron were provided, the lack of some factor in our diets may have been instrumental in preventing the absorption or utilization of this metal.

Since, as far as we are aware, there are no records in the literature of blood studies on dogs fed oatmeal for prolonged periods of time as the sole source of protein, it is not possible to say whether or not the anemia could be attributed to a poor quality or quantity of the protein. This, however, is very unlikely, since the oat protein is biologically adequate for the rat and the adult human.

The relation of the vitamins to blood formation has been investigated from both the experimental and the clinical points of view. Experimentally it has been shown by several observers (Happ, 1922; Cramer et al., 1922; Turner, 1930, and others) that anemia can not be produced in rats on a diet lacking vitamin A. Although Koessler, Maurer and Loughlin (1926) believed that the anemia they produced in rats was due to lack of vitamin A, Simmonds, Becker and McCollum (1927) pointed out that the diet of the former workers was deficient not only in vitamin A but also in vitamin E and iron. Sure, Kik and Walker (1929, 1931) found no noteworthy disturbances of hemopoietic function in rats during avitaminosis–A, but that infection and inanition may complicate the picture during the stage of ophthalmia. No constant changes have been found in the bone marrow of rats fed a vitamin A deficient diet, although in some animals which have survived for a long period of time, there occurs an almost complete replacement of the bone marrow by fibrous tissue (Findley and Mackenzie, 1922). The influence of vitamin A deficiency on the hemopoietic system in man is problematic. However, general nutritional defects, associated with a lack of vitamin A severe enough to produce keratomalacia may be associated with anemia, although numerous instances of the eye condition have been reported without anemia. Keefer and Yang (1929) conclude that, in man, diets deficient in vitamin A may produce keratomalacia without existence of anemia, although the latter may develop from a nutritional defect where vitamin A is only one of the deficient factors.

A number of observers have found that no anemia occurs in rats or in dogs fed diets deficient in the whole vitamin B complex. Sure, Kik and Walker (1929) and Rose, Stucky and Mendel (1930) using rats and Stucky and Rose (1929) using dogs, found that there was actually an increase of hemoglobin which was attributed to anhydremia. However, it should be pointed out that, owing to the early development of polyneuritis, the dogs of Stucky and Rose received the deficient diet for periods considerably

shorter (from 7 to 10 weeks) than those which elapsed before the onset of anemia in our animals (from 11 to 16 weeks) which were receiving adequate vitamin B_1. In man the consensus of opinion seems to support the view that lack of vitamin B_1 does not produce anemia. The absence of anemia in beri-beri was commented upon by Vedder (1913), and Keefer and Yang (1929) in a series of cases of this disease did not find anemia in the majority of patients, although it might be quite marked when, in addition, other nutritional defects are present.

With regard to the effect of deficiency of vitamin B_2, Sure, Kik and Smith (1921) found that in rats a considerable proportion of animals developed a severe anemia, which was especially prevalent in those which had dermatitis. In pellagra (Hillman, 1913) anemia of a variable degree exists, but it is not a prominent feature of the disease, which may be present for a considerable time without leading to anemia. In this connection it is of interest to note that the so-called "pernicious anemia of pregnancy" and "tropical anemia" in India may be cured according to Wills (1931) by either "marmite" or by commercial liver extract,[6] both potent sources of vitamin B_2, while vitamins A and C are ineffective.

Scurvy produced experimentally in guinea pigs is often accompanied by anemia (Meyer and McCormick, 1928). It is well known also that in human scurvy, anemia may occur, which is benefited specifically by the administration of food rich in vitamin C (Mettier et al., 1930). For reasons which have already been mentioned, vitamin C was omitted from the basal diet, and although there were no manifestations or pathological evidence of scurvy in these animals, the possibility is nevertheless to be considered that the anemia may have been due to the lack of this factor.

On account of the marked anorexia which developed in the later stages of the experiments, it may be argued that the anemia could have been attributed so the inanition resulting from a deficient food intake. However it has been pointed out by Benedict (1915) that in man and dogs, starvation does not give rise to anemia. Furthermore, this writer claimed that anemia was not a feature during partial starvation over a long period of time in spite of the development of extreme emaciation. It is not unlikely, therefore, that the anemia in our dogs may have been due to a lack of vitamin B_2 or some other component of the vitamin B complex other than the anti-neuritic.

The knowledge of the nutritional factors determining the level of the lipoids in the blood is scanty, most of the work having been directed toward an attempt to produce a hypercholesteremia in man and animals by food rich in fat and lipoids. Low cholesterol diets have shown variable results

[6] "Hepatopson" and Liver Extract "B.D.H.", both presumably potent for pernicious anemia.

occasionally causing a decrease of the cholesterol level in the blood while in a number of cases no change could be demonstrated.

In view of the low food intake in the later stages of our experiments, it is noteworthy that in starvation or inanition it has been shown that there may be an increase of the fat and lipoids in the blood, followed by a hypocholesteremia when the fat depots are exhausted. This subject has recently been reviewed by Peters and Van Slyke (1931) and Muller (1930). Further, in undernourishment during the World War, Rosenthal and Patrzek (1929) found a hypocholesteremia, while Strathman-Herweg (1920) obtained normal values in undernourished children.

Since there may possibly have been a somewhat low protein content in our basal diet, the work of Barker (1930) concerning the relation between the cholesterol level of the plasma and a disturbed nitrogen balance may be significant. Dogs, fed a diet deficient in nitrogen but otherwise adequate, showed an initial gradual increase followed by a terminal decline in the cholesterol content of the blood.

It has been suggested that the metabolism of cholesterol is influenced by the vitamin A content of the diet (Kimura, 1928; Liang and Wacker, 1925). However, in our experiments, since the lipoid changes in the blood occurred irrespective of the vitamin A content of the diet, the results can not be ascribed to this factor. In pellagra there has been reported hypercholesteremia (Peters and Van Slyke, 1931), whereas lack of vitamin C is said to exercise no influence upon the cholesterol content of the blood in guinea pigs (Moriquand et al., 1925), while in human scurvy the cholesterol level of the blood is below normal (Ssokoloff, 1924).

From the above considerations, although inanition and the absence of vitamin C from the diet can not be definitely excluded as contributory causes in the production of some of the manifestations described, it seems probable that the responsible limiting factor in the basal diet was one or more of the components of the vitamin B complex, other than the antineuritic. However, since the specific effects of deprivation of each of the different components have not as yet been clearly demonstrated in dogs, it is not possible to state to what extent the findings in these experiments may have been dependent on such a deficiency.

SUMMARY

1. An attempt to produce spinal cord degeneration in adult dogs by feeding a diet abundant in cereal and lacking in vitamin A proved unsuccessful. Possible reasons are given for this failure.

2. Irrespective of whether or not the dogs were receiving vitamin A, there occurred, however, a syndrome characterized by anorexia, loss of body weight, dermatitis, skin ulcers, anemia and changes in the concentration of the blood lipoids.

3. It is assumed, therefore, that, in addition to the absence of vitamins A and C, the basal diet was deficient in some other essential factor, which, on indirect evidence, is considered to be probably some portion of the vitamin B complex, other than vitamin B_1.

4. Although administration of additional iron failed to prevent the further development of the anemia, the possibility of failure of absorption or of utilization of this metal is nevertheless to be considered.

5. Continuous heat, as judged by their attitude toward male dogs, was a feature in the two female dogs deprived of vitamin A.

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STUDIES ON INDUCTION OF OVULATION AND THE INHIBITORY INFLUENCE OF CORPORA LUTEA ON OVULATION IN THE RABBIT

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Received for publication January 21, 1932

Bellerby reported in 1929a that ovulation could be induced in rabbits by means of a single intravenous injection of an acetic acid extract of the anterior lobe of the beef hypophysis. In that year also Friedman showed that in the same species ovulation is induced by a single intravenous injection of urine from pregnant women.

The studies which are here detailed were undertaken in the hope of confirming these experiments and of applying them to the solution of certain problems of ovulation and of corpus luteum function. A preliminary report was published in the Anatomical Record (Jares, 1930).

1. Repetition of Bellerby's experiment. Fresh bovine hypophyses were obtained from the slaughter house within a few hours after death of the cattle, and the anterior lobes were shelled out. The original directions of Bellerby (1929b) for the preparation of the acetic acid extract of the anterior lobe of the hypophysis were followed. The resulting extract was clear, light yellow in color, and was non-toxic when administered intravenously in doses as large as 25 cc., but two preparations of this sort failed to induce ovulation. It was thought probable that the potency had been removed by adsorption during the filtration and kaolin treatment called for by Bellerby, and a third extract was made, this time from sheep's hypophyses, employing centrifugation instead of filtration as a means of removing solid debris, and omitting the kaolin treatment. The resulting extract was also clear and non-toxic, and induced ovulation in rabbits when given intravenously in doses of 12 cc.

2. Ovulation induced by alkaline extract. Typical ovulation was also induced in the rabbit by a single intravenous injection of 2.0 cc. of an alkaline extract of whole hypophyses of sheep. This extract was kindly supplied to us by Parke, Davis & Co., and was prepared by the method described by Bugbee, Simond, and Grimes (1931). The potent substance did not dialyze from this extract.

3. Induction of ovulation by pregnancy urine. Friedman's fine discovery was next confirmed (Jares, 1930) by administering the urine of pregnant

women to rabbits intravenously in doses of 5 cc., followed the next day by laparotomy. Ovulation was regularly found to have occurred. This phenomenon has since become well known as the basis of the now widely used rabbit ovulation test (Friedman test) for pregnancy; and even at the time of this first confirmation, it was obvious that the method could be applied to the study of many questions of reproductive physiology. Experiments were undertaken in various directions in the hope of obtaining quantitative data, and of relating the phenomenon in question to the known facts of reproductive physiology in rabbits. As a first step, to determine the minimal quantity of the untreated urine that would induce ovulation in an adult, unmated, and previously isolated rabbit, the dose administered was gradually reduced from the routine 5 cc. until a negative ovulation test was obtained. The first urine specimens that were employed in this work were obtained from a patient 25 years of age and seven and one-half months pregnant. The minimal ovulation dose was found to lie between 0.25 cc. and 0.50 cc., the former dose producing a negative ovulation test, and the latter a positive test.

4. Measurement of inhibitory effect of corpora lutea on induction of ovulation. At this point it became evident that here was a method whereby the supposed inhibitory influence of the corpora lutea on ovulation could be directly tested. If the corpora lutea exert an inhibitory effect upon ovulation, then the minimal dose of pregnancy urine required to produce ovulation should be larger in rabbits having functional corpora lutea in their ovaries than in rabbits containing no corpora lutea. It is evident that experiments of this sort, involving the quantitative dosage of an unknown chemical substance present in unknown concentration in the urine, must be conducted in such a way as to avoid discrepancies due to varying concentration of the urine in different samples, or to other uncontrollable influences such as pathological states of the donors. For this reason the donors were carefully selected, and each group of experiments was carried out by use of a single twenty-four hour specimen.

In a few cases spontaneous ovulation provided the corpora lutea, and in these cases the first dose of pregnancy urine was that given to test the amount required to induce another ovulation in the presence of these corpora lutea. In most of the experiments, however, a first dose of urine was given to induce the formation of corpora lutea, and the dose to be tested was therefore the second.

Corpora lutea thus induced resemble those of experimental pseudopregnancy induced by sterile mating, in macroscopic and microscopic appearance, and also in duration. In all probability they are functionally equivalent to those naturally found. Winter (1931) has demonstrated that corpora lutea induced by an extract of pregnancy urine (Prolan) are able to cause changes in the uterus very similar to those occurring during

pregnancy when the normal corpora lutea are in a functional state; and Friedman (in press) has observed that corpora lutea induced by extracts of the urine of pregnancy are able to stimulate the endometrium with the production of deciduomata after traumatization, just as the naturally-occurring corpora lutea are capable of doing. At the outset of this work, exploration of the rabbits to determine the exact conditions of the follicles, ovaries, and uterus, previous to any experimentation, was performed in each experiment, but since the sexual organs in the majority of cases were in a constant and predictable condition, this preliminary control observation was dispensed with. Besides, the necessary exploratory observations made after the injections disclosed any change that had occurred previously in the follicles, as in the instances of spontaneous ovulation, mating (x-413), and hemorrhagic follicles. No difficulty whatsoever was encountered in readily recognizing the conical, bright-red, freshly ruptured follicles, and the number recorded at the laparotomy performed fifteen to thirty hours after the first injection was completely verified at the second exploration made at a later stage—usually ten days afterwards—by the large and even more easily discernible corpora lutea. Actual extrusion of the ova in these artificially-induced ovulations was proven by washing out the Fallopian tubes with normal saline twenty hours after the injection of pregnancy urine and recovering the ova under the microscope.

Repeated injections of pregnancy urine into the same rabbit cause complications and abnormal conditions, as described below, and therefore the results obtained by the first injection are considered more significant than those following the second, third, and fourth injections, and accordingly have been tabulated separately.

The first group of experiments (tables 1 and 2) was made with urine specimen C, a twenty-four hour specimen obtained from a woman 26 years of age and two and one-half months pregnant. No steps were taken to render the urine aseptic. The total volume was 1215 cc., to which were added 5.0 cc. of chloroform. The specimen was stored in the refrigerator throughout the experimental period.

Reference to table 2, "first injection" portion, shows that 0.05 cc. induced ovulation in rabbits having no corpora lutea in their ovaries, whereas 0.15 cc. was required in rabbits having functional corpora lutea, considering approximately fifty per cent of the cases. Thus a difference of 200 per cent in minimal effective quantities was obtained here. The portion of table 2 wherein all cases are recorded, including the first, second and third injections (into a single test animal) and also the doses diluted up to larger volumes with distilled water, further confirms the results obtained with the first injections recorded in the same table even though complications enter to a greater or lesser degree, and the results are probably not quite as significant as with the first injections. Diluting

the urine with distilled water appeared to reduce its potency somewhat but the findings are rather limited here.

TABLE 1

Urine specimen C—2.5 months' pregnancy stage

NUMBER OF RABBIT	INJECTION NUMBER	URINE INJECTED	DAYS BETWEEN INJECTIONS	NUMBER OF RUPTURES	NUMBER OF CORPORA LUTEA	AGE OF CORPORA LUTEA	NUMBER OF VERY LARGE FOLLICLES	NUMBER OF LARGE FOLLICLES	RUPTURES + VERY LARGE FOLLICLES
		cc.				*days*			
x-281	1	5.0		8			6		14
	2	0.05*	9		3†	9		11	
	3	0.10	5		3†	14	11		
x-282	1	5.0		13					13
	2	0.05*	9		13	9		9	
	3	0.15	5	9	13	14	6	4	
x-283	1	0.30		13			1		14
x-284	1	0.10		5			9		14
x-285	1	0.05		2			11		13
	2	0.05	8		2	8	14	6	
x-286	1	0.025*					13	3	13
x-287	1	0.05*					13		13
x-288	1	0.10		12			1		13
x-289	1	1.0*		12					12
	2	0.15	10	7	12	10	7	6	
	3	0.15	3	4	19	3; 13	2	4	
x-290	1	5.0		21					21
	2	0.05	10		21	10		10	
	3	0.10	3		21	13	8		
x-291	1	5.0		24					24
	2	0.05	10		24	10		14	
	3	0.15	3		24	13	18	4	
x-292	1	5.0		18					18
	2	0.10	10		18	10		14	
	3	0.15	3		18	13	14		
x-293	1	0.025					14		14
x-294	1	0.05		2			9		11
	2	0.10	8		2	8	9		
	3	0.18	4	4	2	12	4	3	
x-295	1	0.10		2			11		13
	2	0.15	8	11	2	8	6	5	
	3	0.15	4		13	4; 12	4	3	
x-296	1	0.05					12		12
	2	0.05	1				9	8	
	3	0.08	3	1			14		
x-297	1	0.10		6			7	1	13

* Diluted.

† One ovary excised.

Taken as a whole, table 2 indicates that the general run of rabbits, unselected except for sexual maturity and isolation, respond with striking similarity to a given amount of the urine both in the normal state, and under the influence of corpora lutea. It seems probable that even greater uniformity of response to a given quantity would have resulted if the urine specimen had been initially freed of certain substances which appeared later and tended to cause some difficulty in maintaining a constant concentration of the active substance in each dose. However, in all these

TABLE 2

Urine specimen C—2.5 months' pregnancy stage

FIRST INJECTION—NO CORPORA LUTEA PRESENT			FIRST INJECTION IN PRESENCE OF CORPORA LUTEA		
Urine	+	−	Urine	+	−
cc.			cc.		
0.025		1	0.05		3
0.05	2	1	0.10		2
0.10	4		0.15	2	
0.30	1				
5.0	5				

All cases, including first, second and third injections and dilutions

WITHOUT CORPORA LUTEA			WITH CORPORA LUTEA		
Urine	+	−	Urine	+	−
cc.			cc.		
0.025	0	2	0.05		5
0.05	2	3	0.10		4
0.08	1		0.15	4	3
0.10	4		0.18	1	
0.30	1				
1.0	1				
5.0	5				

experiments, the urine specimen was adequately agitated immediately before each injection.

About thirty days after the urine specimen had been voided, the minimal ovulation dose under normal conditions increased progressively with the age of the urine, due to, apparently, the deterioration of the active substance. The observations made under the latter conditions are not recorded here. At the onset of deterioration a good working knowledge of the active amounts under the various conditions had been gained, and just when more data on the critical dosages were in order, observations were forced to halt.

A second group of experiments (tables 3 and 4) was performed with

TABLE 3

Urine specimen J—2 months' pregnancy stage

NUMBER OF RABBIT	INJECTION NUMBER	URINE INJECTED	DAYS BETWEEN INJECTIONS	NUMBER OF RUPTURES	NUMBER OF CORPORA LUTEA	AGE OF CORPORA LUTEA	NUMBER OF VERY LARGE FOLLICLES	NUMBER OF LARGE FOLLICLES
		cc.				*days*		
x-401	1	0.10		3	1*	10 (?)	14	
	2	0.30	11		3	11		19
	3	0.50	3		3	14	13	10
	4	0.60	1		3	15	24	
x-402	1	0.05						16
	2	0.08	1					13
	3	0.12	1				13	
	4	0.15	13	3			7	
x-403	1	0.025						13
	2	0.08	1					12
	3	0.15	1				4	10
	4	0.25	13	2	1†	13 (?)	5	1
x-404	1	0.10						12
	2	0.10	27					14
x-405	1	0.15		13				1
	2	0.50	11	18	13	11		2
	3	0.35	10	5	18	10		14
x-406	1	0.12		10			4	
	2	0.40	11	16	12	11		3
	3	0.30	10	7	16	10		11
x-407	1	0.10					17	4
	2	0.15	1				22	
	3	0.20	9	4			14	
x-408‡	1	0.12						13
	2	0.18	1				11	
	3	0.15	9					15
x-409	1	0.10						10
	2	0.15	1					10
	3	0.15	9					12
x-410	1	0.15		4			6	2
	2	0.30	12	5	4	12	1	9
x-411	1	0.12		12			1	1
	2	0.35	11	11	12	11		
x-412	1	0.10						7
	2	0.20	1	2			6	3
x-413	1	0.40		7	9§	17		6
x-414	1	0.12					9	
	2	0.15	9	4				6
x-415	1	0.12		11				5
	2	0.25	9	6	11	9		6

* Resulting from a spontaneous ovulation.

† Probably a luteinized follicle with contained ovum.

‡ Very large animal.

§ Resulting from a normal mating.

TABLE 3—*Concluded*

NUMBER OF RABBIT	INJECTION NUMBER	URINE INJECTED	DAYS BETWEEN INJECTIONS	NUMBER OF RUPTURES	NUMBER OF CORPORA LUTEA	AGE OF CORPORA LUTEA	NUMBER OF VERY LARGE FOLLICLES	NUMBER OF LARGE FOLLICLES
		cc.				*days*		
x-416	1	0.15		8				
	2	0.20	10	6	8	10		5
x-417	1	0.12		1			1	5
	2	0.15	8	8	1	8		1
x-418	1	0.15			9*	10 (?)		13
x-419	1	10.0		17				
	2	0.20	8	10	17	8	1	
x-420	1	5.0		11				
	2	0.15	9		11	9		11

urine specimen J, a twenty-four hour specimen obtained from a woman 18 years of age and two months pregnant. The total volume of the specimen was 1150 cc., to which were added 5.0 cc. of chloroform. The specimen was stored in the refrigerator throughout the experimental period. This specimen was tested for oestrin in spayed rats and proved negative. The difference in the minimal ovulation dose in rabbits having no corpora lutea and in those having functional corpora lutea was not as great with this specimen as with specimen C, being 0.12 cc. and 0.20 cc. (first injections, table 4), respectively, a difference of 67 per cent, considering approximately 50 per cent of the cases. As with specimen C, deterioration began in about thirty days, the minimal ovulation dose increasing and the results becoming somewhat inconsistent. The observations made after the specimen reached thirty days of age are not reported here. Taken as a whole, the data observed with specimen J were not as consistent among themselves as those of specimen C, nor were the differences under the various experimental conditions as great and as definite as with specimen C. This can be explained, partially at least, by greater differences in weight, age and other details than happened to be the case with the animals used with specimen C. Two cases (x-401 and x-417) in which ovulation was induced in the presence of a single corpus luteum with doses smaller than those found necessary to induce ovulation in rabbits having the usual number of corpora lutea, are not included in the summary table (table 4), since there were special circumstances which made them not comparable with the others.

These experiments indicate that in the presence of corpora lutea about three times as much of the pregnancy urine is required to produce ovulation as in the absence of corpora lutea. The most obvious interpretation of this finding is that the corpora lutea exert an inhibitory effect upon

action of the ovulation-inducing hormone. Before accepting this explanation, however, two other possible explanations must be considered.

The possibility that an immunization was produced by an injection of the urine specimen, thereby accounting for the larger amount required to induce ovulation under the lutein condition, was eliminated experimentally: Rabbit x-414 will serve as an example. An initial injection of

TABLE 4

Urine specimen J—2 months' pregnancy stage

FIRST INJECTION—NO CORPORA LUTEA PRESENT			FIRST INJECTION IN PRESENCE OF CORPORA LUTEA		
Urine	+	−	Urine	+	−
cc.			*cc.*		
0.025		1			
0.05		1	0.15		2
0.10		4	0.20	2	
0.12	4	2	0.25	1	
0.15	3		0.30	1	
5.0	1		0.35	1	
10.0	1		0.40	2	
			0.50	1	

All cases, including first, second, third and fourth injections

WITHOUT CORPORA LUTEA			WITH CORPORA LUTEA		
Urine	+	−	Urine	+	−
cc.			*cc.*		
0.025		1			
0.05		1	0.15		2
0.08		2	0.20	2	
0.10		5	0.25	2	
0.12	4	3	0.30	2	
0.15	5	5	0.35	2	
0.18		1	0.40	2	
0.20	2		0.50	1	1*
5.0	1		0.60		1*
10.0	1				

* x-401, see text.

0.12 cc. of specimen J was insufficient in this case to cause ovulation. A second injection of 0.15 cc., nine days after the first, induced ruptures. Furthermore, the massive dose of 10.0 cc. of specimen J was administered to rabbit x-419 resulting in seventeen ruptures, and eight days later, a dose of 0.20 cc. induced a typical ovulation, ten ruptures resulting.

A second possible explanation requires attention. Under the column headed "ruptures plus very large follicles," table 1, there is noted a fairly

constant value, averaging thirteen, except for three cases where large doses of urine were administered and from eighteen to twenty-four ruptures were produced. Evidently, with these large doses, all the follicles attaining even a small macroscopical size are forced to rupture. It might logically be argued therefore that a normally effective dose, administered shortly after a huge one, would find follicular conditions opposed to immediate rupture, and thus fail to induce ovulation. This could be a possible explanation for cases x-290, x-291, and x-292, in which 5.0 cc. were administered in each instance in order to form corpora lutea, and 21, 24, and 18 follicles, respectively, were ruptured. Ten days later, 0.05 cc., 0.05 cc., and 0.10 cc., respectively, was injected, and no ruptures were found in any instance at exploration the following day. However, such conditions could not have obtained, apparently, in cases x-285 and x-294, in which 0.05 cc. was administered at the very first injection, and only two ruptures occurred in both instances, thereby leaving numerous very large follicles for the second test quantities—0.05 cc. and 0.10 cc. respectively—which did not induce ovulation. It should be pointed out here that two separate doses, subminimal for a given animal, were not summated to induce follicular rupture, regardless of the interval—22 hours and upward—between the administrations. Such a case is exemplified by rabbit x-296, whose ovaries contained many very large follicles but no ruptures as a result of the very first injection of 0.05 cc. A second injection of 0.05 cc. administered one day after the first, served to further increase the size of some of the follicles, but produced no ruptures. The sizes of the follicles are not expressed in terms of exact unit diameter, but more generally, since it has been observed that relatively small follicles in one rabbit and relatively large follicles in another, will both rupture in response to the same quantity of active substance.

With both the alternative explanations thus ruled out, it seems probable than an inhibitory effect of the corpora lutea is the actual explanation of the result obtained in these experiments. It was observed in numerous cases that no correlation existed between the number of corpora lutea in an ovary and the number of follicles induced to rupture by the urine injection. When there was a great disparity between the number of corpora lutea in the two ovaries of an animal, the number of newly ruptured follicles in each ovary tended toward equality. It seems therefore that the inhibitory effect upon ovulation exerted by the corpora lutea, acts upon both ovaries, presumably by a humoral mechanism, and is not due to merely local effects.

5. Threshold effect. With the just effective minimal dose the number of ruptures is lowest, while the number of enlarged follicles is generally correspondingly high (table 1), indicating that a certain threshold amount of the stimulating substance is necessary for actual rupture to occur in

any one case. The reverse was observed in those cases where the largest quantities of urine were employed, the number of ruptures being high, and the follicles being few and small. Intermediate doses produced corresponding events, and, in general, the number of ruptures was proportional to the dose up to a certain limit, above which still larger doses did not increase the number of ruptures. Since the above problems are of a statistical nature, and since the data are too few, no graphical representations are herewith attempted.

6. Effect of dosage on the time required for induction of ovulation. In an effort to accelerate the ovulatory process by means of a huge dose of pregnancy urine the following experiment was carried out: Rabbit x-219 received 0.38 cc. of pregnancy urine (4 months stage) intravenously. Rabbit x-233 received 5.0 cc. of the same urine specimen at the same time. Both animals were anesthetized with urethane and explored simultaneously. Care was exercised not to touch the ovaries and warm saline swabs were placed on the exposed tissue during the very brief observational exposures. Actual follicular rupture began in both animals nine hours after injection. At the end of the tenth hour the animal receiving the small dose possessed a total of ten ruptures, 5 large follicles, and three fairly dark blood follicles. At the twelfth hour no further changes could be observed, except that the bright-red orificial plugs were larger and more distinct and the conical shape of the ruptured follicle was more pronounced. The rabbit receiving the large dose possessed a total of five ruptures, and twelve very large follicles at the tenth hour; at the twelfth hour, fifteen ruptures were observed. It is thus inferred that the larger amount of stimulating substance probably produced more widespread follicular development and secretion and more ruptures than the small amount, but that the effective reaction time for rupture was approximately the same in both cases.

7. Ovulation induced during pregnancy. A single observation of induction in a pregnant rabbit by administration of pregnancy urine was made. Rabbit x-413 (table 3) was injected with 0.40 cc. of specimen J on the seventeenth day following mating. Exploration on the eighteenth day disclosed seven ruptures, nine whitish-yellow corpora lutea resulting from the mating, and nine normally developing fetuses. Resorption of the fetuses occurred later, but no inference can be made on a single case as to the significance of this fact.

The observation that the mating of a rabbit during any stage of pregnancy or pseudo-pregnancy does not effect an ovulation (Hammond and Marshall, 1925), while administration of the gonad-stimulating substance induces follicular rupture, suggests that some controlling factor is present in the normal animal. This factor appears to be the inhibitory influence of the corpora lutea, which could effect this result by either depressing the production and secretion of the active substance, or by inhibiting its

functional activity in the follicles. It is possible that the amount of stimulating substance as administered experimentally is too great to be antagonized or that optimal opportunity is not afforded the corpora lutea when the substance is introduced directly into the circulation.

8. Repeated induction of ovulation at short intervals. Ovulation could be induced repeatedly (x-289, x-405, x-406), and whenever desired, by successive injections of pregnancy urine, providing the dose was large enough. By such means rabbits have been kept in a condition of excessive luteinization for as long as seven weeks, at which time experimentation was discontinued. The ovaries became greatly enlarged and monstrously shaped after the first few ovulations, the corpora lutea bulging and crowding in every direction.

9. Experimental production of hemorrhagic follicles. Hemorrhagic follicles were found present at the majority of the exploratory observations. In the case of rabbit x-297, forty blood follicles were found in the two ovaries at the exploration following a first injection of 0.10 cc. of specimen C. No observation as to the number of blood follicles previous to the above injection was made, but their small size and dark appearance indicated that they had been present for some time prior to the above experimentation. However, in other cases, the large, distended, bright-red blood follicles, observed fifteen to thirty hours after the first injection indicated that hemorrhagic follicles may result from administration of the gonad-stimulating substance. Only a few days and two or three doses are required for such a follicle under this forced stimulation to turn from a bright-red color, through red-black, blue-black, and black, and to assume relatively gigantic proportions. A good example of such blood follicles was observed in x-401. These blood follicles occur normally in the rabbit and have been described by Hammond and Marshall (1925). Their similar occurrence under these experimental conditions may possibly indicate that the same processes are involved as under normal environmental conditions, except that the abnormal process as a whole is speeded up under adequate, experimentally-forced stimulation as used here. Those enlarged follicles having only a very slightly bright-red color have been included in the tables as very large follicles and large follicles, depending on their size. However, it should be parenthetically mentioned here that even a recent, true blood follicle was never observed to have ruptured.

10. Observation of the actual rupture of Graafian follicles. Actual rupturing of the follicle was observed many times during the above experimentation. The phenomena occurring during the process of ovulation in the rabbit as observed in these experiments confirm the pioneer studies of Walton and Hammond (1928). In several instances ruptures occurred during exploratory observation as long as seventeen to twenty-six hours after injection. It was also noted during an ovariectomy performed

twenty-six hours after a large injection of pregnancy urine that even gentle handling of the ovary caused several very large follicles to rupture. However, in the above tabulated cases, touching or handling of the ovary itself was rigidly avoided.

DISCUSSION. It is interesting to note that Hammond in 1925 postulated that "the number of follicles which ripen depends upon a limiting amount of *some nutritive substratum* in the blood supply (Heape's 'generative ferment') rather than on any inherent potentiality of the ovary itself," and together with Walton (1928) showed that artificial rupture or ablation of ripe follicles is followed by an immediate compensatory growth of new follicles. The experimental findings recorded above, obtained by quite different methods, bear out and extend these contentions, since follicular growth, maturation and rupture were repeatedly induced at short intervals by the gonad-stimulating substance.

The difference in the minimal ovulation amounts of pregnancy urine in animals with and without corpora lutea appears to lend support to the theory that the corpora lutea inhibit ovulation. However, further general knowledge concerning effective amounts under more varied conditions, and more minute study of the mechanism of the functional activity of the stimulating substance, and also of the inhibiting substance seems necessary before a final conclusion can be made.

Much conjecture and evidence has accumulated regarding the inhibitory influence of corpora lutea on ovulation. However, little of it seems to be free from complications and interfering processes both normal and abnormal. Recently, Patel (1930) has produced evidence that the administration of corpus luteum hormone caused a reduced reactivity of the ovaries of immature mice to the gonad-stimulating substance derived from human placenta, and also probably inhibited the secretion of this potent substance by acting on the pituitary. Wolfe (1931) showed that a much larger amount of the sow's anterior hypophysis tissue in saline suspension was required to induce ovulation in the rabbit when the ovaries of the donors contained active corpora lutea.

This recent, more direct evidence, together with the well-known phenomenon that the squeezing out of the cow's corpora lutea hastens the next oestrous period and ovulation, supports the data reported here, and seems to indicate that there is an antagonism between the corpus luteum hormone and the gonad-stimulating substance or substances of the anterior hypophysis or the similarly acting substance in pregnancy urine.

SUMMARY

1. Ovulation has been induced in the rabbit by a single intravenous injection of the following: 1, an acid extract of the anterior hypophysis (confirming Bellerby); 2, an alkaline extract (Parke, Davis) of the anterior hypophysis, and 3, human urine of pregnancy (confirming Friedman).

2. The minimum ovulation dose (considering approximately 50 per cent of the cases) of untreated human pregnancy urine in normal rabbits varied with the length of gestation of the donor and with the donor as an individual: 0.05, 0.12 and between 0.25 and 0.50 cc. were required from gestational stages of approximately 2.5, 2.0, and 6.5 months, respectively.

3. The minimal dose of untreated pregnancy urine necessary to induce ovulation (considering approximately 50 per cent of the cases) was found to be definitely larger in rabbits whose ovaries contained active corpora lutea. These corpora lutea were experimentally produced by various quantities of untreated urine from the first two human donors mentioned above. One spontaneous ovulation and one fertile mating accounted for the corpora lutea in two additional cases. The minimal ovulation dose in rabbits having corpora lutea was raised from 0.05 to 0.15 cc., a difference of 200 per cent, (employing one preserved 24-hour urine specimen throughout the experimentation), from 0.12 to 0.20 cc., a difference of 67 per cent, (using a single 24-hour specimen).

4. Ovulation in the rabbit has been directly observed. The follicular changes noted in these experiments before, during, and after actual rupture and extravasation of the ovum confirm Walton and Hammond's observations.

5. A massive dose of pregnancy urine does not hasten the rupturing time of about ten hours after administration.

6. Ovulation was induced in a rabbit on the seventeenth day of gestation by injection of pregnancy urine.

7. Changes in the follicle under these experimental conditions appear to be similar to those occurring normally. Hemorrhagic follicles were observed to occur as a result of the administration of anterior hypophyseal substance, and both their number and size were dependent upon the frequency and magnitude of the forced stimulation.

I am greatly indebted to Prof. Karl M. Wilson and the Department of Obstetrics and Gynecology of Strong Memorial Hospital for excellent coöperation in providing the urine specimens and information regarding the donors.

It is a pleasure to acknowledge the interest and assistance of Prof. George W. Corner in these investigations.

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ON THE EFFECTS OF POLARIZATION OF NERVE FIBERS BY EXTRINSIC ACTION POTENTIALS[1]

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Received for publication May 12, 1932

There are several hypotheses assigning important physiological rôles to currents originating in nerve. Among others may be mentioned the theory that attributes impulse propagation to restimulation by an eddying along the fiber of the nerve's action current; the view that Wedenski block is a state of postcathodal depression determined by the potential of the blocked nerve impulse (Erlanger and Blair, 1931b); and the view attributing the relatively refractory phase to the negative phase of the irritability effect determined by the action potential of nerve (Erlanger and Blair, 1931a). These hypotheses predicate action of the nerve's potential on the fiber in which it originates. As instances of suggested extraneous actions of nerve potentials may be mentioned Adrian's view (1930) attributing spontaneous repetitive responses of the fibers of a cut nerve to stimulation by the nerve's demarcation potential, and so-called rheoscopic stimulation.

Our study of the summation and depression intervals determined by brief subthreshold shocks (Erlanger and Blair, 1931a) suggested the idea that such current as leaks from active fibers of a nerve and enters adjacent fibers that happen to be inactive locally must have the effect, just as does a subthreshold shock, of first increasing the fibers' irritability and then decreasing it. In so far as this occurs there must be produced a state tending to bring into phase impulses running slightly out of phase in neighboring fibers conducting at the same rate; it would constitute a mechanism making for the synchronization of action potentials running out of phase in homogeneous fibers of a nerve. It was to ascertain whether there is any such action in normal nerve that this investigation was undertaken. Our results in this respect, however, have proven entirely negative. The experiments will, therefore, be considered in the briefest possible manner.

The *methods* employed involve registration by the cathode ray oscillograph of the amplified action potentials as described in previous publications from our laboratory, except that in our present plant the von Ardenne

[1] This work was made possible in part by assistance from a grant made by the Rockefeller Foundation to Washington University for research in science.

oscillograph[2] replaces that of Johnson. The deflections on the screen of this tube may be made so luminous that they can be directly photographed on sensitive film through a quick lens. The pictures thus obtained are much sharper than our previous contact prints. At the higher driving voltages the von Ardenne tube has about one-fifth the sensitivity of the Johnson tube. To overcome this difference amplification has been more than correspondingly increased. A more complete description of our methods will be given elsewhere.

RESULTS. Mention may be made first of an experiment performed to ascertain whether there is any synchronizing action in normal nerve. The phrenic nerve of the dog is removed from its origin in the neck down to the diaphragm. Two of its roots of origin are placed one each on the terminals

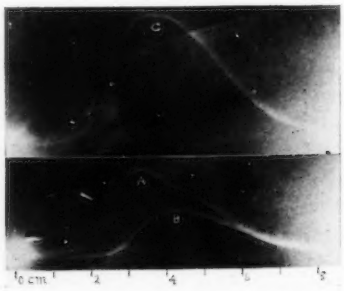

Fig. 1. Submaximal alpha action potentials of the dog's phrenic, each started by stimulation of a different trunk of origin. Conduction 15 cm. *A* and *B* are separate records printed in superposition. *C* is the record of these two action potentials travelling simultaneously but out of phase. Reduced.

of an inductorium and arrangements are made to lead monophasically from the distal end 15 cm. away the action potentials resulting from stimulation of the roots. First, records are made of action potentials slightly above threshold in amplitude from each of the roots separately (see fig. 1). The shocks are so timed that the action potentials they initiate reach the lead at different but known intervals after the spot has started on its horizontal sweep across the screen of the tube. Then the two roots are stimulated during one and the same sweep with some one pair of these stimulation intervals.

It has been found in such experiments that the figure derived from the algebraic sum of the two action potentials running independently invariably is superimposable on the record of the two comparable action potentials running simultaneously but slightly out of phase as summed by the record-

[2] Obtained through the General Radio Company, Cambridge, Massachusetts.

ing mechanism, when an amplitude correction is applied that is necessitated by the characteristic curve of the amplifier (see fig. 1). This temporal agreement of the two sums has obtained no matter what have been the relations of the two action potentials to each other. Since the phrenic roots presumably contain the same kinds of axons and since these presumably are intimately mixed in the nerve trunk the conditions provided in this experiment are regarded as optimal for the manifestation of any extrinsic action fiber potentials may have in normal nerve. Comparable preparations consisting of the sciatic nerve of the bullfrog with two of its plexus trunks likewise have given negative results.

In order to give the leaking currents every possible chance to induce a response the above procedure was repeated on nerves whose reactivity was raised locally by various devices. First it was shown, for purposes of control, that a nerve whose local reactivity is rising at the cathode during the initial stages of the make of a just subthreshold constant current or of a current rising practically linearly at less than the liminal rate does not,

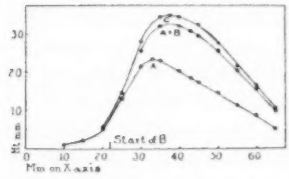

Fig. 2. $A + B$ is the sum of A and B of figure 1; C is C of figure 1. C and $A + B$ differ from each other in respect to height only.

within the limit of error, conduct more rapidly than a normal nerve. But even under these conditions it has been impossible to demonstrate by methods illustrated in figure 1 any influence of the action potentials running in one group of fibers upon the propagation rate of the action potentials running slightly out of phase with them in another group. We conclude, therefore, that *conduction* in a fiber, whether normally or hypernormally irritable, is wholly uninfluenced by currents emanating from its neighbors.

Having failed to demonstrate any effect of extrinsic nerve currents on conductivity we next planned experiments to ascertain whether the current from active fibers affects the *irritability* of neighboring inactive fibers. With our methods it is a simple matter to place an induction shock in any desired phase of an action potential moving along a nerve (Erlanger, Bishop and Gasser, 1926). In tests carried out in this way we have failed to find any enhancement of irritability of inactive fibers exposed to a passing action potential; a just subthreshold testing shock remains so in all phases of a passing submaximal action potential. Even when the irritability of the nerve is raised locally by the procedures mentioned above there is no

stimulation of additional fibers; the current escaping from active axons does not suffice to stimulate neighboring fibers even when they are about ready to fire off.

Neither is the response of a nerve to a just threshold constant current increased by leading into it, through the cathode of the polarizing current, a properly directed maximum monophasic action potential from another nerve; it is not increased even when this action potential arrives at the time the irritability of the nerve being thus polarized is at its maximum.

At this place it may be well to call attention to the fact that when nerve, which is being treated with a just subthreshold constant current, attains the level of maximum irritability and is about to respond an added shock still must be given an appreciable, even though small, intensity if it is to stimulate (Erlanger and Blair, 1931b). Failure of an applied extrinsic action potential to stimulate nerve in that state must, therefore, mean that the stimulating value of the action potential applied is less than that of the shock that is just threshold under similar circumstances. In experiments of this type the leads from the nerves supplying the extrinsic action potential were separated by a distance, about 2 cm., such that they would subtend a very large part of the nerve's potential. The voltage thus derived was not measured. If, however, it be assumed that it was 0.01 volt and that the resistance through which it acted was 100,000 ohms the current applied would have been 0.1 microampere. Under comparable conditions we have found that a rectangular constant current reaches threshold at something less than 0.5 microampere. Since a rectangular current presumably has a higher stimulating value than a current of the shape of an action potential it is fair to conclude that the stimulating values of the extrinsic action potentials have been considerably below that of the threshold constant current. Undoubtedly it is this difference that accounts for the ineffectiveness of the former in these experiments.

In the experiments just described the polarizing leads were on intact nerve surface. The results, as has been said, were negative. Since rheoscopic preparations are known to give positive results experiments were then performed in which the conditions usually obtaining in such preparations were supplied. The method used is illustreatd in figure 3. The preparation consisted of two nerves. First, a constant current was found that was slightly above the threshold of both of the nerves, *A* and *B*. With the arrangement shown in the figure, where the electrodes polarizing *B* were both on intact nerve, the very much submaximal action potential determined in *B* by closure of the constant current was not increased in amplitude by leading the maximum action potential of *A* into *B* even at the moment when the irritability of the latter, as a result of the make of the constant current, was at its maximum. This part of the experiment confirms our previous experience relative to the ineffectiveness of extrinsic

action potentials when applied to intact nerve. Then B was killed at C. Now the addition of the action potential of A materially increased the amplitude of the action potential started in B by the constant current.

It has also been found that positive results are obtained when one of the terminals connecting the extrinsic potential source with the nerve rests on cut branches. With arrangements such as are shown in figure 4, in which the cathode of the polarizing current rests on cut branches, a submaximal action potential started at A and recorded at B increases in height slightly when the action potential is made to pass C at the time the irritability there is rising as a result of the prior closure of the constant current; the effect is maximal at the time the irritability rise due to the closure is at its maximum.

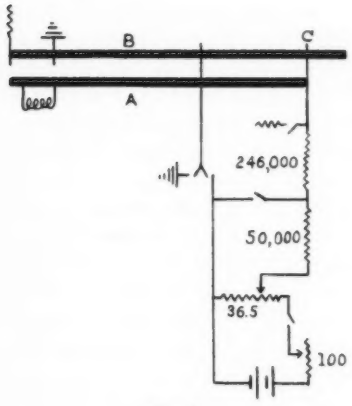

Fig. 3

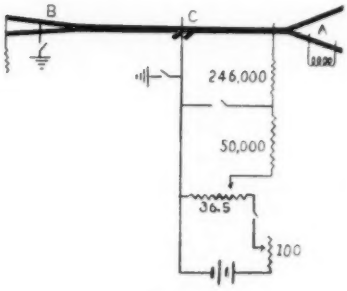

Fig. 4

Fig. 3. Arrangements employed to demonstrate the effect of extrinsic action potentials from one nerve on another nerve, either intact or injured.

Fig. 4. Arrangements employed to demonstrate the stimulating effect of the potentials from one set of fibers upon another at cut branches.

The rôle of the cut end in making effective the current determined by the applied action potential has not been investigated, since we were concerned with the effects developed by action potentials in normal nerve only. It is to be presumed, however, that an important factor in cut nerve is the influence of the open connective tissue and myelin sheaths on the distribution of the current lines determined by the action potential.

The two types of experiments last described were devised as a last resort, for we had reached the point where we began to feel that we could not repeat a simple rheoscopic experiment. Now, however, that we have obtained these positive results we feel justified in concluding that by our

negative experiments we have demonstrated that such current as leaks from fiber to fiber in normal nerve is without physiological significance.

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THE RELATIVE ABSORPTION RATES OF DEXTROSE AND LEVULOSE[1]

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Received for publication March 2, 1932

During a series of studies on closed intestinal loops in dogs it was found that these isolated segments of small intestine with intact mesentery, under favorable conditions became normal in gross and histologic appearance. Preliminary experiments also showed that the loop while in this apparently normal state absorbed quite readily substances injected into it. In order to further test the reliability of the chronic closed loop for absorption studies a series of experiments has been carried out using three methods: *a*, the closed intestinal loop of dogs; *b*, loops of small intestine of anesthetized rabbits; *c*, the gastro-intestinal tract of normal rats. The following report is concerned with the absorption rates of dextrose and levulose. Pfanstiehl sugars with specific rotatory powers were used throughout.

PROCEDURES. a. *Closed loop of small intestine in dogs.* About two weeks after the isolated loop has been made, barring accidents, the animal should be in good condition (1). The loop will have quieted down and the closed ends completely healed. If no fluid is being held in the loop it may be considered to be in normal condition. The skin over the site of the loop attachment to the abdominal wall is cleansed with alcohol and a sterile needle (preferably no. 19, 1.5 inch long) is inserted into the loop. If it has not been washed out for a few days, it should now be washed by gently injecting and aspirating warm physiological salt solution. A loop approximately 12 cm. long may be given 10 cc. of a 20 per cent solution of sugar. One hour after giving the sugar, the loop is aspirated and washed with warm physiological salt solution three times to remove that not absorbed. The total amount of sugar removed is then determined by the Shaffer-Hartman method and the amount of absorption calculated.

b. *Loops of small intestine of anesthetized rabbits.* Young rabbits weighing five to six pounds having been without food for twenty-four hours were anesthetized and the abdomen opened. A section of the ileum about 90 cm. long was ligated at each end and two ligatures about one-half inch apart were placed in the middle of the segment. The sugar solutions were in-

[1] This work was supported in part by Grant 191 of the Committee on Scientific Research of the American Medical Association.

jected with a hypodermic needle and the abdomen closed. The operation was carried out as quickly as possible (approximately five minutes) in order to minimize the length of time the animal remained under the anesthetic. Heat from an electric reflector was then applied and in twenty to thirty minutes the animal appeared to have recovered from the anesthetic. At the end of one hour it was killed by a blow just back of the head. The segments were removed and the contents emptied into beakers. The mucosa was carefully washed with 75 cc. of hot water to remove final traces of unabsorbed sugar.

c. *The rat method* used by Cori (2) was carried out in most respects as indicated in his work. The last food was given forty-eight hours before the experiment but water was left in the cage during this period. The cages had screen bottoms to allow feces to drop out of reach of the animals. The weights of the rats varied between 160 and 310 grams. By means of a no. 8 French catheter 2 cc. of a 50 per cent solution of the sugar were introduced into the stomach. After the injection the amount of sugar remaining in the catheter was determined. At the end of the absorption period the animal was killed by a blow on the head. The abdomen was immediately opened and ligatures placed around the esophagus just above the cardia and around the ileum near the ileo-colic valve. It was found unnecessary to include the lower bowel. After removal, this part of the gastro-intestinal tract was thoroughly washed by forcing 75 cc. of warm water through it in three separate portions. After making the fluid content and washings up to 100 cc. the amount of sugar was determined.

The amount of the sugar absorbed was arrived at by accurately estimating the amount placed in the intestinal tract or segment thereof and subtracting from this the amount of sugar recovered. Normax glassware checked against United States Bureau of Standards certified burettes was used entirely. The sugar solutions were made by weight and after twenty-four hours checked in the polariscope. In the dogs and rabbits the solutions were introduced with a syringe after being measured out with accurate pipettes. The amount of sugar remaining in the syringe was determined on several occasions and found to be less than 30 mgm. Since this amount was uniform for the two sugars it was left out of the calculations. In rats where a small catheter was used the amount of sugar remaining in and sticking to the outside of the catheter was determined in each case. Where this amount was above 50 mgm. it was subtracted from the estimated amount given. In all experiments the total amount of sugar recovered was diluted to 100 cc. in a volumetric flask and a one to fifty dilution was made of this for Shaffer-Hartman determination. Further care was taken to adjust the dilutions when indicated to bring the final reading in cubic centimeters of thiosulphate used for titration within the limits of 12 to 18 cc. since this has been shown to be the amount of sugar most accurately determined by

this method. The amount of protein in the recovered specimens was found to be a negligible factor in the sugar estimations. A study of the Shaffer-Hartman method on known dilutions of levulose has shown that the method gives results 10 per cent too low. This has also been taken into account in the levulose determinations.

The amounts of absorption of levulose and dextrose in one hour when 10 cc. of a 20 per cent solution (2 grams) were injected into closed loops of dogs were studied. The average of 19 separate experiments on eight different dogs was 0.86 gram for dextrose; 26 experiments on eight different dogs using levulose was 0.79 gram. In these experiments as in those to follow, it is considered that the loops were approximately at the same level in the ileum and near the same size. While some small differences may have existed, the use of the animal for both sugars would tend to exclude these as factors that would alter the conclusions.

TABLE 1

Results of repeated experiments on individual dogs expressed as grams of sugar absorbed by closed loops in one hour from 10 cc. of a 20 per cent solution of the sugar

DOG 61		DOG 132		DOG 135		DOG 131	
Levulose	Dextrose	Levulose	Dextrose	Levulose	Dextrose	Levulose	Dextrose
1.15	0.98	0.72	1.38	0.90	1.03	0.61	1 01
0.81	0.97	0.81	1.11	0.76	0.97	0.63	0.97
0.86	0.99	0.86	0.74	0.71	0.72	0.60	0 85
		0.93	0.75	0.70	0.73	0.67	0.77
		0.66	0.65			0.60	0.93
						0.59	
						0.56	
0.95	0.98	0.80	0.91	0.77	0.86	0 61	0 90

When 10 cc. of a 10 per cent solution of sugar was given the average absorption for dextrose (5 expts.) was 0.52 gram and for levulose (12 expts.) 0.46 gram. Another series of seven experiments with each sugar (20 cc. of a 10 per cent solution) gave an average of 0.91 gram for dextrose and 0.95 gram for levulose.

The best comparison of absorption rates would obtain where a series of experiments is carried out on a given dog alternating the sugars as the series progresses. Any changes in the loop or in the condition of the dog is thus reflected equally upon the two sugars. In table 1 are shown the results from experiments on four dogs. Dog 131 seemed to show a distinct preference for dextrose while the other animals showed a tendency to absorb dextrose only slightly more rapidly.

The first series of rat experiments was carried out upon rats varying in

weight from 160 to 310 grams. These were all healthy animals but obtained from different sources, consequently their previous diet may have varied. Periods of 30, 60, 90 and 120 minutes were used. The average absorption of levulose in each of these intervals was less than that of dextrose. The greatest difference was in the two-hour period and amounted to 19 per cent. However, in view of a seemingly consistently slower rate for levulose absorption, it was decided to run a longer series on a group of rats of the same stock that had been kept on a uniform, adequate diet. These were all young rats with weights varying from 185 to 300 grams.

In this series twenty-one rats were given levulose and twenty-four dextrose. The absorption period was one hour. The greatest amount of levulose absorbed was 0.70 gram; the least 0.41 gram. The greatest amount of dextrose absorbed was 0.80 gram while the least amount was 0.35 gram. The average amount of levulose absorbed per rat was 0.55 gram; and of dextrose, 0.57 gram. It would seem that if any marked difference in the rates of absorption of these sugars by rats exists, these experiments would have shown it.

In 36 rabbit experiments using an absorption period of one hour the above results were confirmed. This method is less desirable because of the effects of the anesthesia than either of the other methods. When 5 cc. of a 10 per cent solution were given the average absorption was 0.29 gram for dextrose and 0.28 for levulose. Further results were obtained on giving 1 gram of the sugar (some were given 10 cc. of a 10 per cent solution and others 20 cc. of a 5 per cent solution). The average absorption was 0.46 gram dextrose and 0.44 gram levulose. The sugars were alternated with respect to "high" and "low" positions in the ileum and the averages of the lengths of loops used for the two sugars were approximately equal.

Discussion. Different investigators have reported that levulose has a slower absorption rate than dextrose (3), (4). Their work has hardly been carried out under conditions that would permit of definite conclusions. The more recent work of Cori where rats were used would seem less open to criticism. He found that if the absorption rate of dextrose be taken as 100, that of levulose is 43. This remarkable difference was explained by Cori on the basis of stereoisomerism. Our results show no such difference in any of the methods used. There is considerable variation in the amounts of absorption in all series. This has been the experience of other investigators. Cori drew his conclusions on the amount of absorption of sugar the first hour by averaging the results of five experiments. In the case of levulose the maximum and minimum amounts of absorption varied by 38 per cent, yet the average of these was carried through for estimation of absorption during the second, third, and fourth hours in different groups of rats. Only by increasing the number of rats in a series could this obvious possible source of error be eliminated. From a series

of 37 rats given levulose we found the average amount of absorption for one hour to be 0.50 gram; from a series of 40 rats given dextrose the average absorption was 0.55 gram (the two series taken together). This is a difference of 9 per cent. In a series of this length, where care has been exercised to overcome all possible sources of error, averages should be indicative of the relative rates of absorption.

In agreement with the findings of Pierce, Osgood and Polansky (5), our results show no relationship between body weight of the rat and absorption. In many instances we observed that a young rat weighing 160 grams would absorb more sugar in one hour than an older rat weighing over 250 grams. The average results of our experiments on rats show a decided decrease in absorption the second hour. The fastest rate of absorption for each sugar is seen in the first thirty-minute period. However, our series is not sufficiently long to make any quantitative statement as to the relative amounts of sugar absorbed during the first and second thirty-minute periods.

CONCLUSIONS

1. Chronic closed loops of ileum in the dog may be used to study absorption. In some respects this method is more dependable than other methods now in use.

2. The three methods used here are in agreement with respect to the relative rates of absorption of dextrose and levulose.

3. Dextrose is absorbed slightly more rapidly than levulose. There is some indication that individual animals may differ in their ability to take up levulose.

4. The rates at which the two sugars are taken up decrease with time. In rats the most rapid absorption takes place during the first thirty-minute period.

5. Our findings show no consistent relationship between body weight of the rat and the amount of absorption over a given time.

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IS LEVULOSE CONVERTED TO DEXTROSE IN THE PROCESS OF ABSORPTION FROM THE INTESTINE?[1]

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Received for publication March 2, 1932

In their studies on hepatectomized animals Bollman and Mann (1931) made the interesting observation that levulose is less effective in preventing the symptoms of hypoglycemia than dextrose. In order that levulose might be effective it was necessary that administration be begun early and larger amounts be given than when dextrose was used. Its efficiency depended upon its conversion to dextrose. It was further found that when the gastro-intestinal tract was removed together with the liver, levulose was without effect in preventing the symptoms of hypoglycemia. The authors made the logical inference that the intestinal tract is capable of bringing about the conversion of fructose to dextrose.

While studying the absorption of sugars from chronic closed loops of ileum in dogs, it occurred to us to further investigate the question of the conversion of fructose to dextrose by the intestinal mucosa. Either by examining specimens from the loop after injecting levulose or by studying the blood from the mesenteric vein from the loop while absorbing the fructose solution definite information should be obtained.

In order to determine amounts of levulose in the presence of glucose the colorimetric method of Corley (1929) was used. This method was found to be quite reliable in determining dilutions up to 0.005 per cent. Where possible, both the polariscopic and Shaffer-Hartman methods were used to further check the findings. When using the Shaffer-Hartman method in the presence of levulose results are somewhat inaccurate. On pure levulose solutions of known concentration this method of determination has been found to give figures under the actual amount by about 10 per cent.

The first observations were made on the aspirated material from loops one hour following the injection of 10 cc. of a 20 per cent solution of levulose. A specimen was used for determining the total reducible sugar by the Shaffer-Hartman method, a cleared specimen was used for a polariscopic reading, and a levulose estimation made by Corley's method. The results of these findings did not indicate the presence of dextrose in the

[1] This work was supported in part by Grant 191 of the Committee on Scientific Research of the American Medical Association.

loop fluid. However, this does not exclude the possibility of the process taking place in the mucosa at the moment of absorption of the molecule. The only way to investigate this possibility was to study the mesenteric blood from the loop during the process of absorption. The dog was given 10 cc. of a 20 per cent solution of levulose via the closed loop. Forty-five minutes later the animal was anesthetized with ether and the abdomen aseptically opened. The mesenteric vein from the loop was exposed and 10 cc. of blood withdrawn. A similar amount of blood was taken from the

TABLE 1

Total reducible sugar and levulose determinations on systemic and mesenteric blood samples one hour after the injection of 10 cc. of a 20 per cent solution of levulose into closed loops in dogs

DOG NUMBER	TOTAL BLOOD SUGAR, SHAFFER-HARTMAN		LEVULOSE IN BLOOD, CORLEY METHOD	
	Mesenteric blood	Systemic blood	Mesenteric blood	Systemic blood
84	0.215	0.203	0.013	None
92	0.214	0.212	++	None
49	0.269	0.237	0.018	None
89	0.213	0.212	+	None
131	0.210	0.185	0.009	None
132	0.210	0.200	0.010	None

TABLE 2

Total blood sugar and levulose determinations on the bath in which a living segment of rabbit's small intestine containing 3M/4 solution has been kept for one hour

LENGTH OF LOOP	AMOUNT OF SUGAR	BATH	TIME	SHAFFER-HARTMAN	LEVULOSE (CORLEY)
cm.	*cc. 3M/4*	*cc.*	*minutes*		
12	3.2	65	60	0.065	0.055
10	3.0	50	60	0.020	0.024
12	4.0	50	60	0.032	0.032
11	3.0	50	60	0.058	0.060
11	3.0	50	60	0.046	0.050
11	3.0	50	60	0.059	0.050

heart. The abdomen was closed and the animal allowed to recover. Total blood sugar and levulose determinations were made on both blood samples. Because of the anesthetic the blood sugar ran high but that is considered of no significance in regard to the levulose content. Table 1 gives the results of experiments on six dogs.

In each experiment the mesenteric blood sugar was greater than that of the systemic blood. Although the method for levulose determination gives fairly accurate results to 0.005 per cent no experiment showed levulose in the systemic circulation. That the sugar was being absorbed is shown by the fact that levulose was present in the mesenteric blood from

the loop. Of course it might be possible that the sugar could be absorbed faster than the conversion took place. The evidence in these experiments, however, would not tend to indicate that such was occurring. The difference between the amount of sugar in the mesenteric and systemic blood samples was in most instances similar to the amount of levulose found in the mesenteric blood.

These results were supplemented by work on isolated segments of small intestine of rabbits. Anchinachie, Macleod and Magee (1930) have shown that living segments of small intestine suspended in isotonic saline solution exhibit the property of selective absorption. Fresh segments were taken from young animals (weight 5 to 6 pounds) and after ligating one end over a rubber covered lead weight and the other over a suitable glass tube 8 to 10 cm. long, the segment was suspended in oxygenated Ringer's solution kept at 37°C. The lead weight was just sufficient to keep the segment immersed in the Ringer's solution. The sugar solution (3M/4) was added via the glass tube. All segments remained active throughout the experimental period of one hour, at which time the bath was examined for total sugar and levulose content. The results are shown in table 2. In four of the six experiments the levulose concentration was found to be as high as the total sugar. If any levulose had been converted to dextrose in passing through the intestinal wall the total reducible sugar would have been greater than the amount of levulose found.

SUMMARY AND CONCLUSIONS

Solutions of levulose were introduced into chronic closed loops of ileum in dogs and into isolated living segments of rabbits' small intestine kept in oxygenated Ringer's solution at 37°C. No glucose was found in the loop fluid after exposure to the mucosa for one hour. The Ringer's solution in which the living segment containing levulose was suspended for one hour showed levulose but no glucose.

Under anesthesia laparotomy was performed upon six dogs and blood taken from the mesenteric vein from the closed loop while absorbing levulose. In all experiments levulose was found in the mesenteric blood while samples of heart's blood taken at the same time showed none.

The evidence obtained from these experiments indicates that levulose is normally taken up by the intestinal tract as such. However, this work does not exclude the possibility that under circumstances such as existed in Bollman and Mann's experiments, the power to convert levulose to dextrose might be called into play.

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PHYSIOLOGICAL REVIEWS

Contents of Volume XI, 1931

An Index to Volumes I to X inclusive was published in the January issue

Contents of Volume XII, 1932